THE SYNTHESIS OF PRECURSORS AND CHIRAL INTERMEDIATES OF LIPOIC ACID

by

David Anthony Howes

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DECLARATION AND ACKNOWLEDGEMENTS

This thesis is a record of research work carried out in the Department of Chemistry and Molecular Sciences at the University of Warwick, during the period from October 1977 to September 1980. It has been presented for no other degree, and is believed to be wholly original except where due reference is made.

I should like to thank the Chairman for making the laboratories of the Department available, and all the other members of the Department who helped during the three years.

I acknowledge a maintenance grant from the Science Research Council in conjunction with Wellcome Research Laboratories, Beckenham, Kent. I am grateful to the latter for providing facilities for two most enjoyable summers of experience in industrial research and especially to Dr Alan Hudson who supervised my work there.

I would like to express my deepest gratitude to Dr Bernard Golding for his invaluable help and encouragement and unfailing enthusiasm.

I would also like to thank Mrs C.A.M. Billing for typing the thesis and finally a special thanks to my wife for courage in the face of chemistry and for her help with some of the typing.

IN MEMORY OF MY FATHER

ABSTRACT

This thesis describes the synthesis of optically active precursors and intermediates to enantiomers of lipoic acid - a biologically important molecule present in almost all living organisms.

Chapter 1 comprises an up-to-date review of the information gained about lipoic acid since its discovery in 1951. Various syntheses of racemic (including radio labelled) lipoic acid are included. The medicinal use of lipoic acid, its chemotherapeutic potential, biological function and a possible role in oxidative phosphorylation are also discussed.

In Chapter 2 the reasons for, and objectives of, the work are stated and the rationale used in designing the various syntheses is examined. The concept is introduced of making the lipoic acid skeleton by coupling a four carbon protected carboxylic acid capable of formation of a terminal carbanion with a 4-carbon optically active 1,2-oxirane containing a terminal hydroxy group. Suitable starting materials are listed for the oxirane and means of protecting the carboxyl functions are discussed. A strategy for the synthesis of (R)- and (S)-8-alkyl lipoic acids is also outlined.

Chapter 3 examines the potential of 1-methyl-2,6,7-trioxabicyclo [2,2,2]octanes as carboxyl protecting groups. Although a number of derivatives were successfully synthesised and showed ideal properties for the protection of carboxylic acids, the 4-haloalkyl derivatives were inert to carbanion formation. As 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane easily formed a Grignard reagent which readily reacted with oxiranes it was decided to use it as the acid containing fragment in the

synthesis of (R)- and (S)-lipoic acid (the acid function being generated by hydrolysis of the dioxolane group and oxidation of the resulting methyl ketone).

Chapter 4 describes the synthesis of (R)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane, a precursor to an optically active oxirane of use in the synthesis of (R)- and (S)-lipoic acid. (2R,3R)-Di-n-butyltartrate is the starting material used and the optical purity of an intermediate, (R)-1,2-dihydroxybut-3-ene is determined.

Chapter 5 contains details of the synthesis of (S)-(2-benzyloxyethyl)oxirane from (S)-malic acid via the intermediate (S)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane (the (R)-isomer of which was obtained in Chapter 4 from (2R,3R)-di-n-butytartrate). The optical purity of (S)-(2-benzyloxyethyl)oxirane is determined. The remainder of the Chapter deals with the successful coupling of (2-benzyloxyethyl)oxirane with the Grignard derivatives of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane to give the lipoic acid skeleton in optically active form. Preliminary experiments for the remainder of the synthesis of (S)-lipoic acid are then outlined. An alternative route was not successful as an intermediate proved difficult to react with the Grignard derivative of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane.

Chapter 6 covers an attempt to synthesise an optically active oxirane from (S)-methionine. (S)-4-Methylthiobutane-1,2-diol is successfully synthesised but attempts to convert it into the oxirane derivatives failed and led to an unexpected product, (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide. This compound exhibited some interesting chemical

properties but initial attempts to obtain the oxirane derivative from it by treatment with base were unsuccessful.

In Chapter 7 a proposed route to (R)- and (S)-methyllipoic acid from (R)- and (S)-ethyl lactate is outlined. The critical stereoselective reduction of an hydroxyketone intermediate is investigated using 4-hydroxy-2-oxopentane as a model. A method of investigating the nature of the product from reducing 4-hydroxy-2-oxopentane is developed and used for a number of different reducing agents.

ABBREVIATIONS

b.p.	Boiling point
9 BBN	9-Borabicylo[3,3,1]nonane
DMF	Dimethyl formamide
DSS	3-(Trimethylsilyl)-1-propanesulphonic acid sodium salt hydrate
DMSO	Dimethyl sulphoxide
DNP	Dinitrophenylhydrazone
g	Gram
h	Hour
i.r.	Infra red
M	Molarity
mg	Milligram
ml	Millilitre
mol	Mole
m.p.	Melting point
min	Minute
nm	Nanometer
n.m.r.	Nuclear magnetic resonance
r.b.	Round bottom
r.t.	Room temperature
TMS	Tetramethylsilane
THF	Tetrahydrofuran
t.l.c.	Thin-layer chromatography
u.v.	Ultra violet
v.p.c.	Vapour-phase chromatography

CONTENTS

CHAPTER 1

LIPOIC ACID: A REVIEW

α-Lipoic acid is a naturally occurring molecule present in most microorganisms, plants and animals and essential for many enzyme-catalysed reactions.

1.1 DISCOVERY

The existence of α-lipoic acid was recognised by a number of laboratories leading to different and confusing trivial names based on the biological response observed.

In 1937[1,2], Snell observed that both sodium acetate and extracts of natural materials, whilst not essential, stimulated the growth of lactic acid bacteria. The work was continued in 1946 by Giurard, Snell and Williams[3] who investigated the role of acetate used in the growth medium of bacteria and found that it served as a buffer as well as a precursor to various lipids. It was further shown[4] that yeast extract had a growth-promoting effect on *Lactobacillus caesi*, 440 times greater than the sodium acetate in the medium. The unidentified active component of the yeast extract was termed 'acetate-replacing factor'.

An independent observation was made in 1941 by Dewey[5] who reported that the ciliated protozoan, *Tetrahymena geleii* would only grow in caesin medium supplemented with crude fractions of natural materials. Crude extracts of natural materials were found to contain two growth factors, one water-soluble, and the other precipitated from aqueous solution. These were designated factors I and II, respectively. By 1945, Kidder and Dewey[6] had identified

factor I as purine and folic acid and in 1949[7] a highly active unidentified substance was isolated from factor II and given the tentative name of protogen. Chromatographic techniques demonstrated that protogen exists naturally in at least two forms, protogen-A and protogen-B.

O'Kane and Gunsalus in 1947[8] reported that *Streptococcus faecalis* (strain 10C1) cells grown on a synthetic medium were not able to oxidise pyruvate until a small amount of a heat-stable factor present in yeast extract was added. Subsequent detailed reports by Gunsalus and co-workers[9,10] described an assay method by measuring oxygen-uptake manometrically and denoted the active principle as 'pyruvate oxidation factor'.

In 1949 Snell and Broquist[11] used concentrates of protogen and pyruvate oxidation factor in place of acetate for the culture of *L. casei* and other lactic acid bacteria. They found that the growth promoting action of the 'acetate replacing factor' was duplicated. It was concluded that 'acetate replacing factor', 'protogen' and 'pyruvate oxidation factor' were identical.

1.2 ISOLATION

The isolation of a pure sample of the biologically active factor from natural materials, was undertaken by Reed *et al.* in 1951[12]. Yeast extract was analysed by paper chromatography and growth factors were detected by placing the chromatogram onto an agar plate seeded with lactic acid bacteria. After 20 minutes the paper was removed and the agar plate incubated. Zones of growth on the 'autobiograph' indicated the presence of a growth factor. In this way five growth factors were found to be present in yeast extract. The multiplicity of growth factors in yeast led

Reed to use a new extraction procedure[13]. He found that acid hydrolysis of yeast extract produced a fraction of only two components but doubled activity. Alternative sources of the two component factor were investigated, the best being the acid hydrolysis of the water-insoluble residue of beef liver. Partition chromatography on an alkaline-buffered silica gel column resulted in a separation of the two components, both of which appeared to be acids, one less polar than the other. A 16,000-50,000 purification was achieved to give a preparation containing 15% of the less polar acid.

Gunsalus *et al.*[14] independently recognised the existence of a weak or slightly polar acid and a strong or more polar acid in hydrolysed yeast and liver extracts. The two substances were separated, by countercurrent distribution between organic solvents and aqueous solutions at various pH values. A 10,000-fold purification was obtained giving a concentrate containing approximately 3% of the less polar acid.

A collaborative programme was undertaken by workers at the University of Texas, the Clayton Foundation of Research, the University of Illinois and Eli Lilly and Company, to isolate a pure sample of the less polar acidic component of liver extract[15]. Attention was diverted to this constituent for two reasons. Firstly, the crude acid fraction was found to contain twice as much less polar acid than polar acid. Secondly, the observation of Reed *et al.*[13] that the latter was produced from the former during chromatographic manipulation indicated that the polar component was not naturally occurring. The collaborative research resulted, in the Spring of 1951, in the isolation of a single, pure crystalline compound from liver which exhibited the activity of protogen-A, pyruvate oxidation factor, and acetate replacing factor. The compound was called lipoic

acid due to its acidic nature, high solubility in non-polar solvents and its ability to catalyse the formation of a precursor of fatty acids *via* oxidative decarboxylation of pyruvate to acetate. A prefix, α, was given to lipoic acid, not to be used in the chemical sense but to distinguish it from the more polar component of liver extract, which was entitled β-lipoic acid. The isolation of 30 mg crystalline α-lipoic acid from 10 tons of liver residue represented a 600,000-fold concentration of active material. Shortly after this achievement the isolation of pure β-lipoic acid was reported[7,6,17]. Treatment of liver residue with papain, an enzyme, gave a 'digest' which was autoclaved with sodium hydroxide and then treated with acid. Counter-current distribution and chromatography on silica gel afforded a yellow oil from which a pure crystalline S-benzylthiouronium salt was obtained.

With the isolation of α- and β-lipoic acid in pure forms attention was focussed on the elucidation of their structures.

1.3 STRUCTURE ELUCIDATION

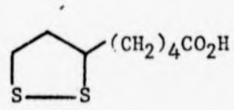
Analytical data on lipoic acid were obtained simultaneously and independently by Reed *et al.*[18-21] and Brockmann *et al.*[22,23] in the early fifties. The experiments and results carried out by the two groups were identical and are summarised as follows: the shape of the titration curve of crystalline α-lipoic acid indicated the compound to be a monocarboxylic acid of pKa 4.7. This was confirmed by a band in the i.r. spectrum at 1740 cm^{-1} due to an aliphatic carboxyl group. The pKa value implied that polar or unsaturated groups were not present at positions α- or β- to the carboxyl group. Spot tests showed the presence of sulphur. Elemental analysis and molecular weight determinations by electrometric titration established the

molecular formula as $C_8H_{14}O_2S_2$. The existence of conjugated double bonds was ruled out by the absence of a relevant peak in the ultraviolet spectrum. A negative nitroprusside test implied the absence of thiol groups, whereas a positive result after α-lipoic acid was treated with sodium cyanide, suggested the existence of a disulphide bond. The polarographic half-wave potential and hydrogen ion reduction potentials corresponded to those of a cyclic disulphide. The carbon skeleton of lipoic acid was shown to be a straight chain because desulphurisation with Raney nickel gave octanoic acid.

Both groups came to the conclusion that α-lipoic acid is a cyclic disulphide containing a straight chain of eight carbon atoms with a terminal carboxylic acid group. The i.r. spectrum of α-lipoic acid did not show a band at 2960 cm^{-1} which signified the absence of a C-methyl group in the molecule. It was thus concluded that one of the sulphur atoms must be linked to the terminal carbon atom of the skeleton. The evidence led Reed and Brockmann to the same conjecture, that α-lipoic acid is the cyclic disulphide derived from either 4,8-, 5,8-, or 6,8-dithiooctanoic acid (1, 2, 3, Fig. 1.a). Brockmann gave the name of thioctic acid to the series of compounds to denote the sulphur containing acid of eight carbon atoms. The number of the carbon atom to which the secondary sulphur atom is attached was used as a prefix to designate each member of the series, i.e. 4-, 5-and 6-thioctic acid.

Establishment of the position of the secondary sulphur atom on the carbon skeleton by means of degradation studies was not feasible with the small amounts of lipoic acid available by isolation procedures. Although liver residue is one of the richest sources of lipoic acid, 1 g of it contains only 1.6-3.2 μg of the active material. For this reason attempts to confirm the structure of

FIG. 1.a



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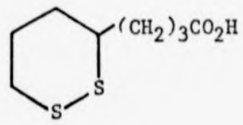

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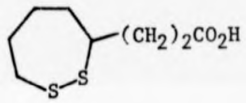

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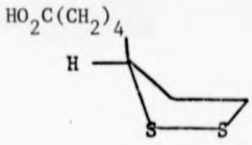
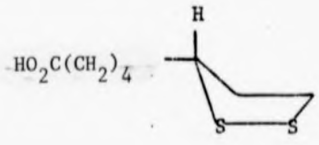
FIG. 1.b



S-Lipoic Acid



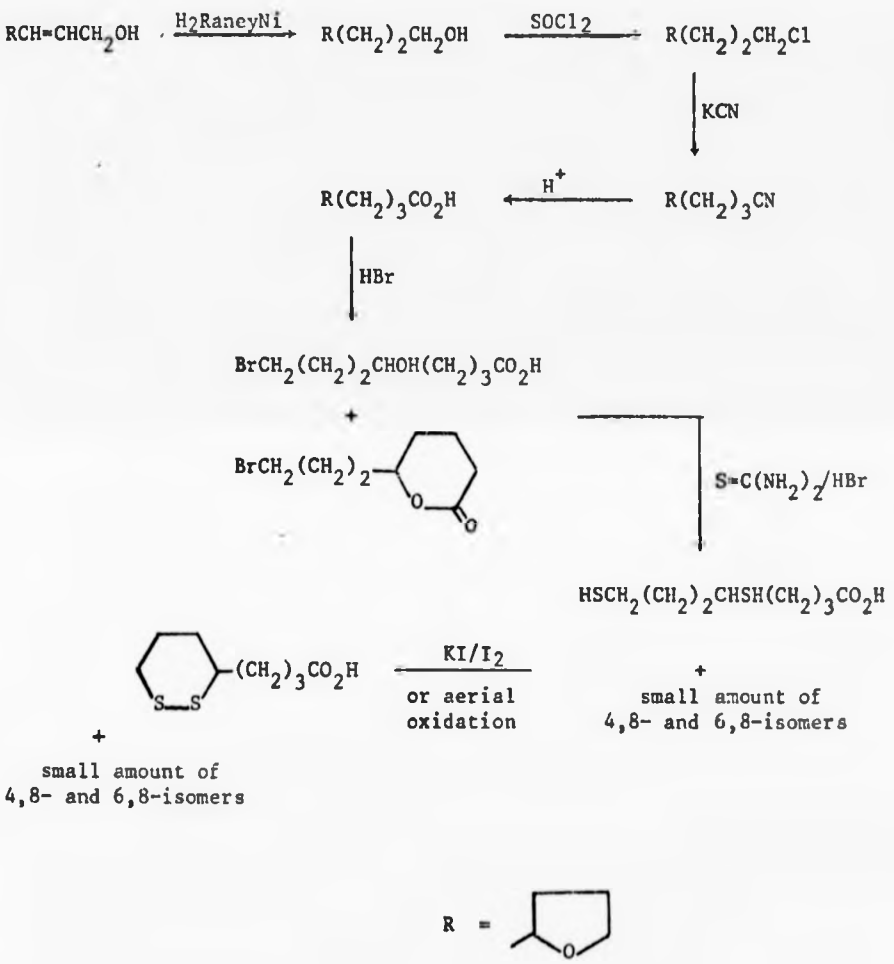
R-Lipoic Acid

α-lipoic acid by synthesis of all three proposed compounds were made.

1.4 PROOF OF STRUCTURE BY SYNTHESIS

Brockmann and his co-workers reported a synthesis of α-lipoic acid[27] at the end of 1951. Shortly afterwards in 1952 Reed, and his co-workers, published a similar synthesis[24] (Scheme 1.1). Both syntheses started from 4-(2-tetrahydrofuryl)butanoic acid obtained from furyl acrolein by hydrogenation of the double bond, replacement of the hydroxy group with chlorine and nucleophilic substitution with cyanide ion. Hydrolysis of the resulting 4-(2-tetrahydrofurylbutyronitrile) gave the corresponding acid. The tetrahydrofuran ring of the 4-(2-tetrahydrofuryl)butanoic acid was cleaved with hydrogen bromide to give a mixture of 8-bromo-5-hydroxy octanoic acid and its lactone. The product was refluxed with thiourea and hydrobromic acid to give, on hydrolysis of the thiouronium salt, 5,8-dimercaptooctanoic acid with smaller amounts of the 4,8- and 6,8- acids. A mixture of isomers was obtained owing to migration of the hydroxyl group of 8-bromo-5-hydroxyoctanoic acid upon treatment with acid. Oxidation of the dimercaptooctanoic acids with potassium triiodide (potassium iodide/iodine) by Brockmann and his co-workers, and aerial oxidation by Reed, *et al.* gave a yellow oil which was presumed to contain the 4-, 5- and 6-membered ring disulphides, and found to have 20% of the activity of pure α-lipoic acid isolated from liver residues. Reed[25] and his co-workers isolated a pure substance from the mixture which had identical properties to that of naturally occurring α-lipoic acid. Brockmann and co-workers, however, isolated all three isomeric disulphides and found that the 5-membered ring cyclic disulphide was 1000 times more active than

SCHEME 1.1

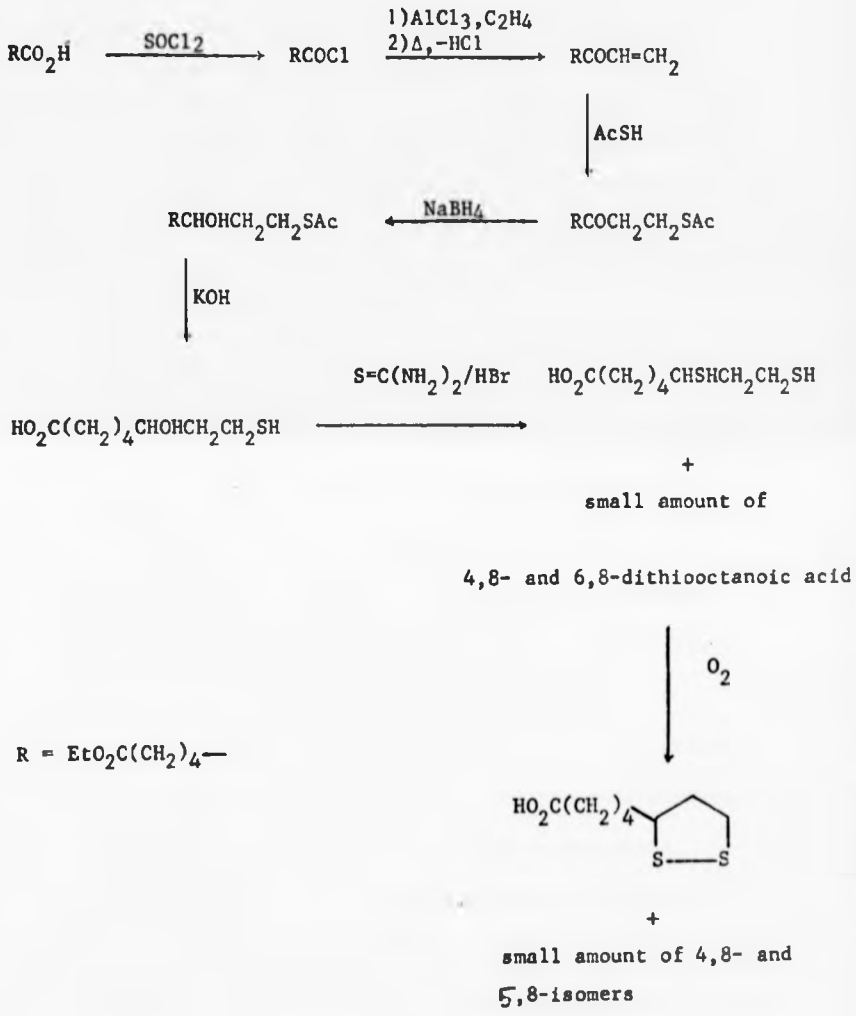


Synthesis of 4,8-, 5,8- and 6,8- Lipoic Acids
from Furyl Acrolein

the 4-or 6-membered rings[26]. The definite structure of α-lipoic acid was confirmed by Brockmann's synthesis of the 6,8-lipoic acid by a different route[27]. This (Scheme 1.2) involved an aluminium chloride-catalysed addition of ethyl adipoyl chloride to ethylene and elimination of HCl to give ethyl-6-oxooct-7-enoate Addition of thioacetic acid to the alkene group of this acid gave ethyl 8-acetylthio-6-oxooctanoate which was reduced to the corresponding hydroxy ester. Basic hydrolysis yielded 6-hydroxy 8-thio octanoic acid which was converted to a mixture of 4,8-, 5,8- and 6,8-dithiooctanoic acid by reaction with excess thiourea in hydrobromic acid. The major product was the 6,8-isomer and 6,8-thioctic acid was obtained by bubbling oxygen through an aqueous potassium carbonate solution of the dithiol. The synthetic material was identical in every respect to α-lipoic acid obtained from biological materials.

Further evidence for the structure of α-lipoic acid was presented by Calvin and his co-workers[28,29] who used 6,8-lipoic acid as a model for the conversion of light into energy in photosynthesis. It was noted that 6,8-lipoic acid exhibited an absorption maximum in the u.v.region at 330 nm (ε = 150), which had been overlooked previously due to its weakness. It was known that as the ring size of cyclic disulphides increases the absorption maximum is displaced to a shorter wavelength. The absorption of lipoic acid was therefore shown to be a characteristic of a 1,2-dithiolane ring since its absorption maximum occurs at a wavelength corresponding to 5-membered ring disulphides. Thus, the structure of α-lipoic acid was accepted as 1,2-dithiolane-3-pentanoic acid (1, Fig.1.a).

SCHEME I.2



Confirmation of the Structure of Naturally Occurring Lipoic Acid by the Synthesis of 6,8-Lipoic Acid from Ethyl Adipate Acid

1.5 NOMENCLATURE FOR LIPOIC ACID

Throughout the discussion, the active component of liver extract now known to be 1,2-dithiolane-3-pentanoic acid, has been referred to as acetate replacing factor, protogen-A, pyruvate oxidation factor, 6-thioctic acid, 6,8-lipoic and α-lipoic acid. Although many of these names are still in use, in order to maintain clarity in the rest of this thesis, 1,2-dithiolane-3-pentanoic acid will be called simply, lipoic acid.

1.6 RATIONALE OF LIPOIC ACID SYNTHESES

All syntheses of lipoic acid proceed *via* 6,8-dithiooctanoic acid. This compound, known as dihydrolipoic acid, was originally oxidised to α-lipoic acid by air or potassium triiodide, but the use of oxygen and a catalytic amount of iron (II) chloride proved to be the better method. Dihydrolipoic acid is very often derived from octanoic acids substituted in the 6- and 8-positions with combinations of chloride, bromide, hydroxyl, ether or ester groups. Treatment of these groups with a thiourea/hydrobromic acid mixture gives a thiouronium salt which can be hydrolysed to the thiol. Thiocyanate, thiosulphate and thioacetate have been reacted with halo and hydroxyl groups of 6,8-disubstituted octanoic acids to give intermediates which, on hydrolysis, yield dihydrolipoic acid. Sodium sulphide and sulphur have been used toconvert 6,8-dihalo and 6,8-dihydroxy octanoic acids directly to lipoic acids. Thio-ethers can be derived from the reaction of benzyl mercaptan with the tosylate of an alcohol or by treatment of a halide with the sodium salt of benzyl mercaptan. Conversion of 6,8-dithio-ethers of octanoic acid to dihydrolipoic acid has been accomplished by hydrogenolysis or by reduction with sodium in liquid ammonia. In lipoic acid

syntheses, octanoic acids carrying hydroxyl groups in the 6- and 8-positions are often converted to the corresponding bromide or chloride by treatment with phosphorous tribromide or thionyl chloride, respectively. The halides offer a cleaner route to dihydrolipoic acid than direct transformation of the dihydroxy to the dithiol compound. If an alcoholic group is present at the 6-position of an octanoic acid the compound is very often used in its lactone form and converted to dihydrolipoic acid with thiourea and hydrogen iodide.

1.7 SYNTHESES OF LIPOIC ACID

The first syntheses of lipoic acid were developed during the elucidation of its structure. The syntheses using tetrahydrofuryl-butanoic acid[21,24] were tedious and led to a mixture of isomers, the main product being the wrong one. The method of Brockmann[26] (Scheme 1.2) using ethyl adipoyl chloride had the advantage of a readily available starting material and gave 6,8-lipoic acid as the major product, but the overall yield from ethyl adipoyl chloride was only 8%.

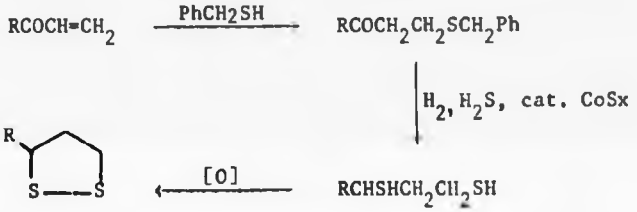
Reed and Nui in 1955[30] published a synthesis of α-lipoic acid with a relatively high overall yield of 36%. The route was a modification of Brockmann's synthesis. The intermediate ethyl 8-chloro-6-oxooctanoate was reduced with sodium borohydride and the resulting hydroxy group was converted to chloride. The 6,8-dichloro-octanoic acid obtained was converted to lipoic acid by standard reactions. Soper *et al.*[31] reported another modification of Brockmann's route. Benzyl mercaptan was added to ethyl 6-oxooct-7-enoate to give ethyl 8-benzylthio-6-oxooctanoate. The oxo group of this compound was converted directly to a thiol by treatment with hydrogen,

hydrogen sulphide and cobalt sulphide catalyst. Under these conditions the benzylthio group was reduced to the thiol (Scheme 1.3). Unfortunately, a large amount of polymeric by-products were produced resulting in a lower yield than in the original synthesis. Further modifications using methylmercaptan in place of benzyl mercaptan gave low yields.

Octanoic acid precursors to lipoic acid have been synthesised by chain elongation of hept-6-enoic acids (Scheme 1.4). Addition of formaldehyde[32,33] or acetic acid/sulphuric acid[34] to hept-6-enoic acid gave a mixture of 6,8-methylenedioxyoctanoic acid and 6,8-diacetoxyoctanoic acid which was converted to 6,8-dihydroxy octanoic acid by treatment with sulphuric acid and methanol and then to lipoic acid by standard methods. The disadvantage of this route is the lengthy preparation of hept-6-enoic acid from either tetrahydrofuran *via* 1-bromo-4-chlorobutane and hept-6-ynoic acid or tetrahydrofuryl-alcohol by way of 5-bromopent-1-ene.

The carbon chain of lipoic acid has been built up by alkylation of the enamines of suitable ketones[35-37]. Thus, the pyrrolidine enamine of cyclohexanone was reacted with bromoacetic acid to give an 8-carbon oxo ester. The ketone was protected and the ester reduced with $LiAlH_4$ to give, upon deprotection, and acetylation, 2-(2'acetyoxyethyl)cylcohexane. Peracid oxidation of this compound gave the 1 → 6-lactone of 6-hydroxy-8-acetoxyoctanoic acid, which upon treatment with thiourea/HI, then hydrolysis and oxidation, gave α-lipoic acid (Scheme 1.5). The advantages of this synthesis were the easily available starting material, inexpensive reagents and simple reactions involved. The yield was claimed to be 19% from cyclohexanone. A similar synthesis has been reported[38] from cyclopentenylenamine by reacting it with β-butyloxypropionyl chloride. Peracid oxidation again led to the formation of a lactone,

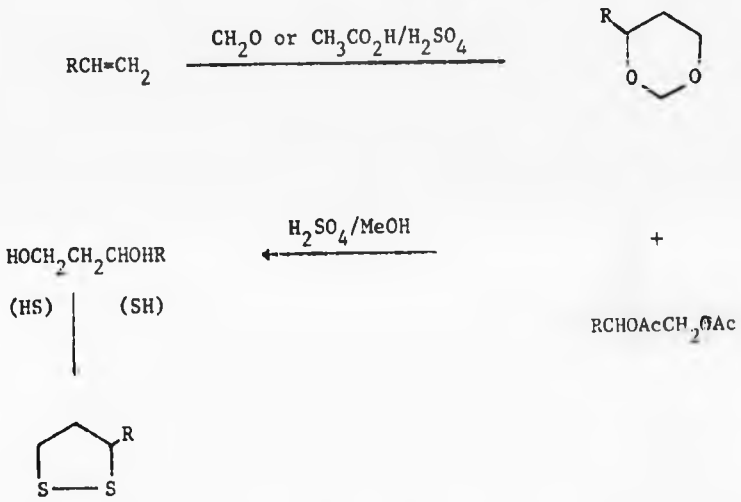
SCHEME 1.3



R = $EtO_2C(CH_2)_4$—

A Modification of the Synthesis of
6,8-Thioctic Acid from
Ethyl 5-Oxo hept-6-enoate

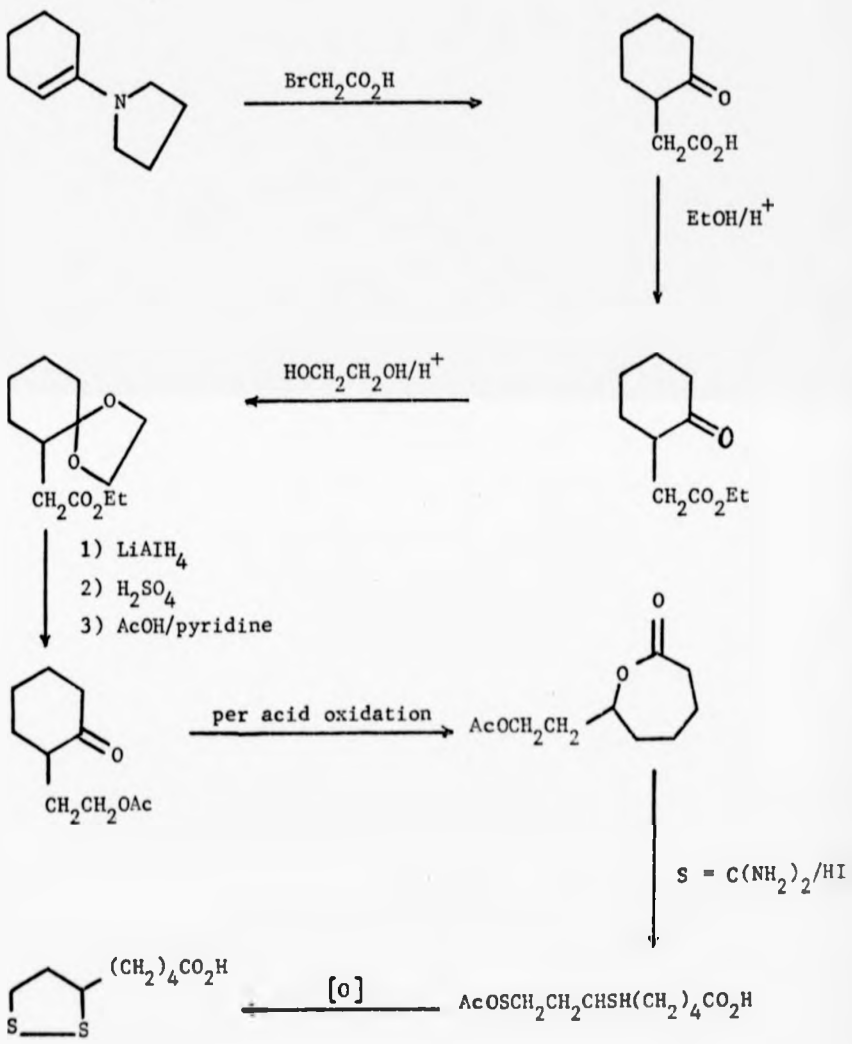
SCHEME 1.4



R = $HO_2C(CH_2)_4-$

Synthesis of Lipoic Acid by Chain Elongation of Hept-6-enoic Acids

SCHEME 1.5



Synthesis of Lipoic Acid from the Pyrrolidene Enamine of Cyclohexanone

easily convertible into lipoic acid.

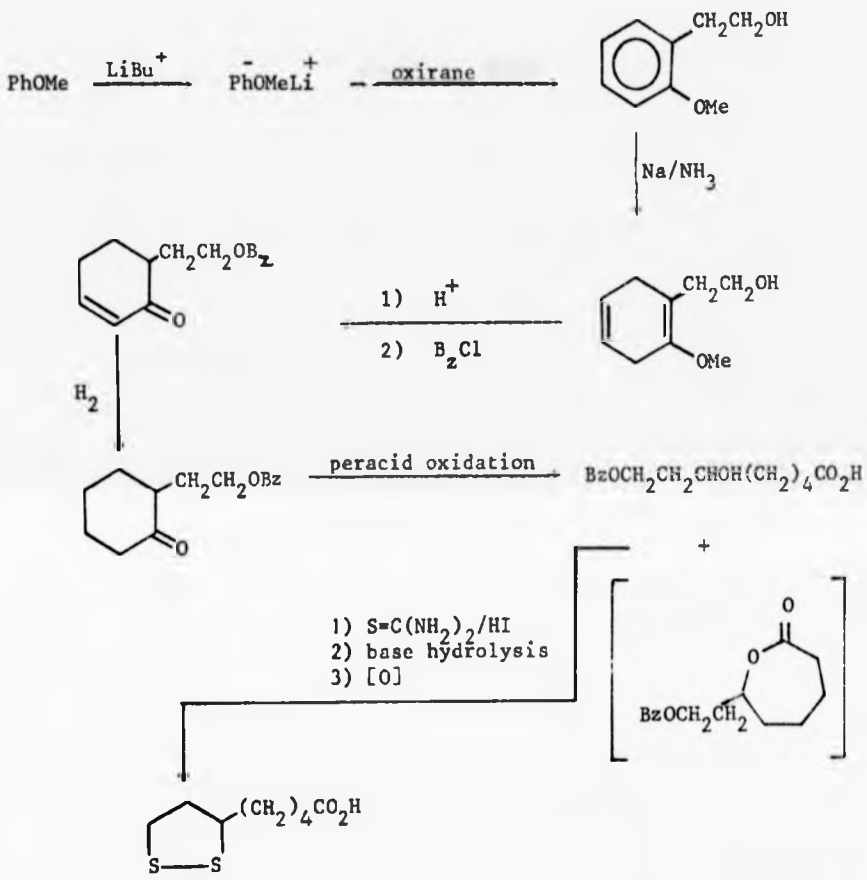
A very simple synthesis of lipoic acid, starting from the readily available anisole[39] was reported in 1962 (Scheme 1.6). Metallation of anisole with butyl lithium followed by reaction with oxirane gave 2-(2-hydroxyethyl)anisole. Birch reduction resulted in the formation of the dihydro compound which was benzoylated to the ester. Mild acid hydrolysis produced the conjugated ketone which was reduced by catalytic hydrogenation to the saturated ketone. Peracid oxidation converted the ketone into a mixture of 8-benzoyloxy-6-hydroxyoctanoic acid and a smaller amount of lactone. Treatment with thiourea and hydroiodic acid, followed by base, gave dihydrolipoic which was oxidised to α-lipoic acid.

The most recent synthesis of lipoic acid was reported in 1978[40]. Acetic acid was reacted with butadiene [catalysis by Pd(II)] to give a 3-acetoxy octa-1,7-diene telomer. The terminal double bonds of this substance were hydroborated with diborane producing octan-1,6,8-triol after treatment of the intermediate alkylborane with alkaline hydrogen peroxide. Protection of the 6- and 8-hydroxy groups allowed oxidation of the remaining hydroxy group with Jones' reagent (chromium trioxide/H_2SO_4). Deprotection afforded 6,8-dihydroxyoctanoic acid which was esterified and converted to lipoic acid by treatment with thiourea/HI, followed by hydrolysis and oxidation (Scheme 1.7).

1.8 MANUFACTURE OF RS-LIPOIC ACID

Of the syntheses discussed so far none can compete on a large scale with Reeds and Nius' synthesis using addition of ethylene to ethyl adipoyl chloride to form 8-chloro-6-oxooctanoate. The starting material is cheap and the dichlorooctanoate intermediate is easily made and cleanly converted to lipoic acid with sodium

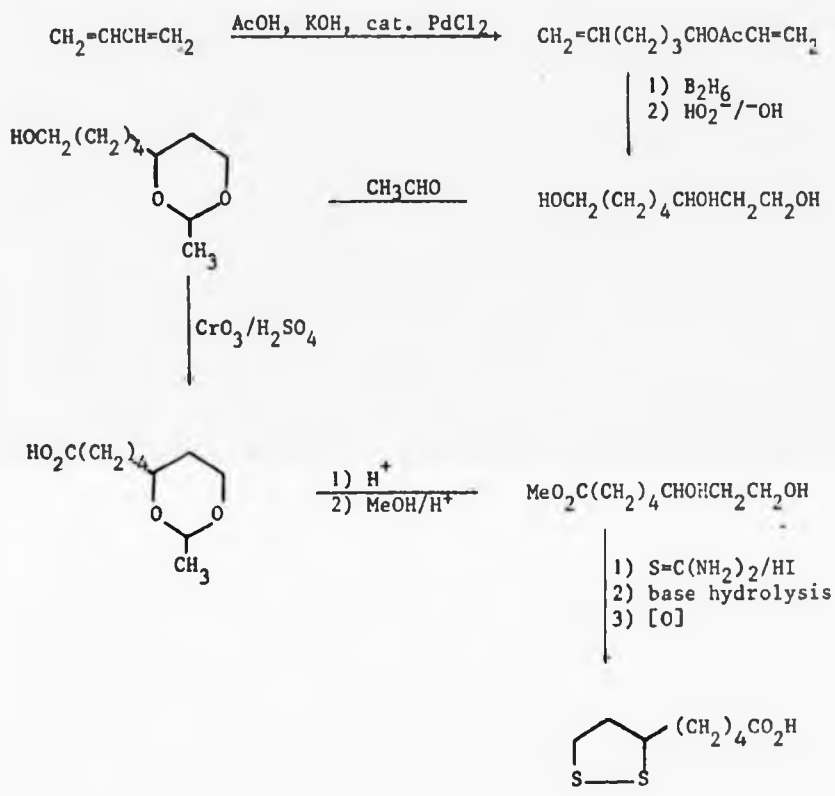
SCHEME 1.6



Bz = benzoyl

Synthesis of Lipoic Acid from Anisole

SCHEME 1.7



Synthesis of Lipoic Acid from a Telomer of Butadiene

benzyl mercaptide. Even so, many syntheses[41-45] involve the elimination of HCl from 8-chloro-6-oxooctanoate to give 6-oxooct-7-enoic acid. Addition of alcohol or carboxylic acids followed by catalytic hydrogenation produces ethers and esters of 6,8-dihydroxyoctanoic acid which can be readily transformed into lipoic acid on treatment with HI/thiourea. Dihydrolipoic acid can be obtained directly from 6-oxo oct-7-enoate by treatment with H_2S/H_2 in the presence of MoSx[46] under vigorous conditions, but this method is rarely used on a large scale due to formation of polymeric disulphides. Many large scale syntheses utilise the addition of ethylene to ethyl adipoyl chloride. Treatment of the reaction product, ethyl 8-chloro-6-oxooctanoate with alcohol gives 8,8-alkoxy-6-oxooctanoic acid. This compound is then hydrogenated to form 8-hydroxy-6-hydroxy octanoic acid whose esters or lactones readily yield dihydrolipoic acid on treatment with thiourea/HI.

1.9 SYNTHESIS OF OPTICAL ISOMERS OF LIPOIC ACID

No route has been established for the direct synthesis of the optical isomers of lipoic acid, nor has lipoic acid itself ever been successfully resolved. Optically pure material is available either from liver residue or by the resolution of a racemic intermediate during some stage of its synthesis. The preparation of the optical isomers of lipoic acid was first reported by Walton *et al.* in 1954[47,48]. Thioacetic acid was added to 7-carbethoxyheptenoic acid to give RS-3-acetylthio-7-carbethoxyheptanoic acid. Treatment of this compound with (-)-ephedrine gave a crystalline salt corresponding to the levorotatory form of lipoic acid. (+)-3-Acetylthio-7-carbethoxy-heptanoic acid was regenerated from the non-crystalline (-)-ephedrine salt and was purified through its benzhydroxylamine salt. In this way 30% of the dextrorotatory and 20% levorotatory 3-acetylthio-7-

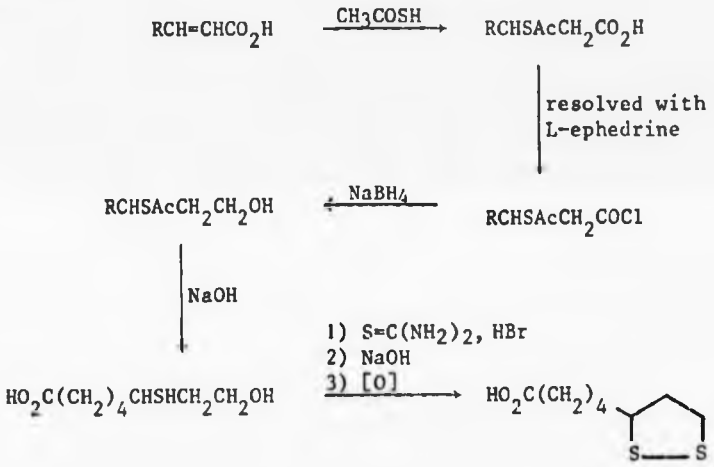
carbethoxyheptanoic acid was isolated from the racemic mixture. The optically pure acid obtained in this way was treated with thionyl chloride and the resulting acid chloride reduced with sodium borohydride to the corresponding alcohol. Hydrolysis of the ester gave 8-hydroxy-6-thiooctanoic acid, which was converted to lipoic acid by standard procedures (Scheme 1.8). Although this procedure gave optically active α-lipoic acid, its use as a viable synthesis was subjugated by difficult, lengthy synthesis of the starting material from monoethyl adipate. The 7-carbethoxyhept-2-enoic acid could only be purified as the diacid which had then to be re-esterified. Also, overall yields of lipoic acid were extremely low, approaching 1%.

In 1957 Acker and Wayne[49] resolved 6,8-dichlorooctanoic acid, an intermediate in Reed and Nius' synthesis of lipoic acid, through its ephedrine salt. Fractional crystallisation gave pure (+)-enantiomer in 48% yield and 16% of a mixture of both enantiomers. Lipoic acid itself was resolved by fractional crystallisation of its cinchonidine salt but only in yields of 0.5-4% in total and again the levorotatory isomer proved to be very impure.

1.10 ABSOLUTE CONFIGURATION OF LIPOIC ACID

The absolute configuration of (+)-and (−)-lipoic acid has not been proved conclusively, although Mislow and Meluch[50] produced some evidence to suggest that the dextrorotatory isomer may have R-configuration (Fig. 1.b). (+)-3-Acetylthio-7-carbethoxyheptanoic acid, an optically active intermediate in Walton's synthesis of lipoic acid was converted to 3-thioloctanedioic acid by treatment with sodium hydroxide. (+)-Thioloctanedioic acid was found to form a continuous series of solutions of different composition upon heating with the solid crystalline (+)-3-methyloctanedioic acid. However,

SCHEME I.8



R = $EtO_2C(CH_2)_4$—

Preparation of Optical Isomers of Lipoic Acid by the Resol ion of -3acetylthio-7-carbethoxyheptanoic Acid

(-)-3-thioloctanedioic acid and (+)-3-methyloctanedioic acid were immiscible under the same conditions. It was concluded that the configuration of (+)-3-thioloctanedioic acid and (+)-3-methyloctanedioic acid were the same and since the latter was known to possess an S-configuration it was proposed that (+)-3-thioloctanedioic acid and thus lipoic acid possess R-configuration.

1.11 SYNTHESIS OF RADIOLABELLED LIPOIC ACID

The synthesis of lipoic acid labelled with sulphur-35 of high specific activity was undertaken in order to provide material for tracer studies in biological systems.

In 1955, Adams[51] and Reed and Thomas[52] introduced sulphur-35 into the 6,8-dibenzylmercaptooctanoic acid intermediate of Reed and Nius' synthesis of lipoic acid by reacting benzyl mercaptan-S^{35} with 6,8- dibromooctanoate. Benzyl mercaptan-S^{35} was prepared by reacting benzyl magnesium chloride or bromide with amorphous sulphur-35.

Later Acker and Wayne[49] obtained lipoic acid of high specific activity directly by the reaction of 6,8-dichlorooctanoic acid with sodium sulphide and sulphur-35.

1.12 CHEMISTRY OF LIPOIC ACID

(a) Physical Properties

RS-Lipoic acid crystallises in the form of pale yellow platelets, melting at 60-61°. The crystalline substance is soluble in benzene, ethyl acetate and methanol, sparingly soluble in petroleum ether and insoluble in water.

(R)-Lipoic acid exists as yellow platelets with a melting point of 46-48°. The highest, measured, specific rotation is

104^{o} (c 0.88, benzene).

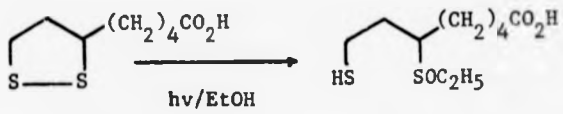
(S)-Lipoic acid crystallises as blunt yellow needles melting at $47-52^{o}$ C. The highest measured specific rotation is -113 (c 1.88, benzene). Optical purities have not been measured for the resolved enantiomers of lipoic acid.

(R)-Lipoic acid has double the biological activity of racemic lipoic acid and (S)-lipoic acid is inactive.

(b) Photochemistry

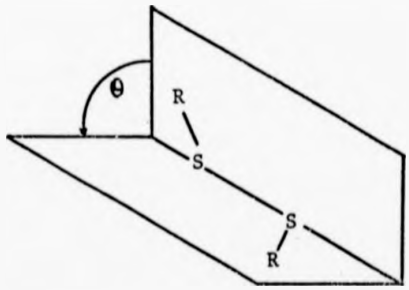
Barltrop *et al.* reported that photolysis of 1,2-dithiolane in neutral solution with near ultraviolet light resulted in polymerisation. In acidified ethanol the dithiolane ring was destroyed but no polymerisation occurred. On this basis, it was suggested that dithiyl radicals produced by photolysis reacted with the solvent to produce an unstable thiol sulfenate (Fig.1.c). However, the nature of this reaction was not elucidated. Whitney and Calvin[53] photolysed lipoic acid and upon examination of the resulting u.v. spectrum found a definite set of products had been formed, but they were not identified. Walton *et al.*[48] found that the u.v. spectrum of solutions of lipoic acid changed considerably on exposure to ordinary light for two days. It was postulated that linear disulphide polymers were formed once the disulphide bond was broken, but again no product studies were made. Brown and Edwards[54] photolysed lipoic acid in different solvents and detected products by t.l.c. and u.v. spectroscopy. Products were formed in each solution, the number and type depending on the solvent. The chain length was determined by the solvent and the length of the polymer decreased with increased availability of protons in the solvent. A mechanism was proposed in which the disulphide bond was broken homolytically in the first step to give a biradical. The reaction could then proceed by attack of the biradical on other lipoic

FIG. 1.c



The Photolysis of Lipoic Acid

FIG. 1.d



Dihedral Angles (θ) in Aliphatic Disulphides

acid molecules to form long chains. Transfer of protons from the solvent to the radical chain was thought to be responsible for the formation of shorter chains. In the solid state lipoic acid is relatively stable to either heating or exposure to light. The inference is that only when the crystal lattice is broken is the 1,2-dithiolane ring able to open homolytically to a dithiyl radical.

(c) Oxidation and Reduction

During the course of the work on the isolation of lipoic acid the tendency of it to undergo oxidation to a sulphoxide (β-lipoic acid) in t presence of oxygen or peroxides was noted. Which sulphur atom is oxidised has not been established, but it is assumed to be the one at C-8 of the carbon skeleton since the specific rotation of the sulphoxide prepared from (S)-lipoic acid is almost identical with that of the latter compound. If the sulphur atom at the C-6 asymmetric centre were oxidised, a marked change in optical properties might be expected. Lipoic acid is reduced readily to 6,8-dithiooctanoic acid by sodium borohydride or zinc and hydrochloric acid.

(d) Spectroscopic Properties

In comparison to open-chain disulphides, the ultraviolet absorption of 1,2-dithiolanes is strongly displaced toward longer wavelengths. The angle θ (Fig. 1.d) which the two R-S bonds form in strain-free open-chain disulphides is about 90°. Incorporation of the disulphide group into a carbocyclic ring without deformation of the interplanar angle of 90° is possible only in large rings. With the decreasing number of ring members (7 → 6 → 5) θ becomes smaller, and orbital energies change. This variation has been studied by Bergeson[55] who considered the S-S chromophore as an isolated π-electron system and its excitation as a transition from a nonbonding $\pi(3p\pi)$ level to a nonbonding $\sigma(\sigma 3p)$ level. Because of the different symmetries

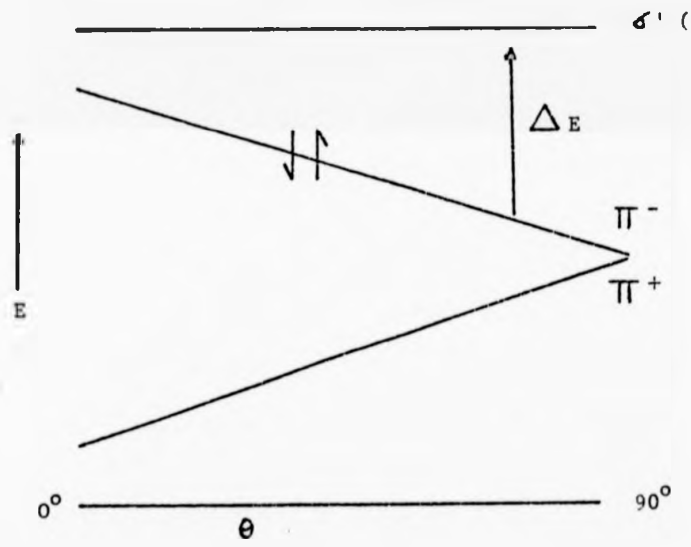
of the π and σ-levels with respect to the S-S axis Bergeson concluded that the energy of the ground state, but not that of the excited state, must depend on the dihedral angle (Fig.1.e). Thus, as ring size of cyclic disulphides decrease, θ decreases, and ΔE decreases with a resulting shift to longer wavelength of the maximum in the electronic spectrum.

The i.r. spectrum of lipoic acid in the NaCl region has been reported[25] and peaks assigned to the carbonyl, hydroxyl, and various C-H vibrations. The KBr i.r. spectrum of lipoic acid has also been investigated[56], but the sulphur-sulphur bond is ill-defined and difficult to characterise due to low intensities (small changes in the dipole moment upon stretching of the group). Similarly, the carbon-sulphur bonds, appearing in the same region of the spectrum have not been assigned unambiguously. The sulphur-sulphur bond is best studied using Raman spectroscopy since it appears as a strong band due to its lack of dipole moment. Lipoic acid has not yet been examined by this technique.

(e) Reactivity

The biological activity of lipoic acid depends on the reactivity of the carbocyclic disulphide bond. This reactivity was first interpreted[57] as due to the high strain energy of the five-membered ring, but subsequent thermochemical measurements showed its strain to be comparable with straight chain disulphides, about 3.5 kcal/mol[58]. Lipoic acid reacts with radicals, electrophiles and nucleophiles. Thus, it exhibits a pronounced tendency to undergo radical polymerisation upon heating on exposure to light, facile oxidation with electrophilic agents such as persulfate[59] and ready reaction with nucleophiles such as RS^- and CN^- [27]. The reaction of lipoic acid with radicals and electrophiles has been explained[60]

FIG. 1.e



Dependence of Excitation Energy ΔE on the Interplanar Angle θ in the Electronic Spectra of Aliphatic Disulphides

as a consequence of the small interplanar angle of 27°. The full p_z orbital of one sulphur atom is therefore not planar with the empty d orbital of the neighbouring sulphur atom and cannot form a π-bond. A result of this is that the electrons of the p_z orbital are available for electrophilic or radical attack. In addition to this a more important factor is thought to be the much lower entropy of activation for a bimolecular reaction on a relatively rigid ring than reaction on a flexible chain.

Nucleophilic reactions of lipoic acid have received very little attention. However, Fava *et al.*[59] have shown that activation energies for the reaction of 1,2-dithiolane and dibutyl disulphide with butanethiolate differ only slightly. They concluded that the reactivity of the 1,2-dithiolane ring must be due to a much lower activation entropy.

1.13 PHARMACOLOGY

An international symposium[61] in 1955 established lipoic acid as a pharmacologically useful compound with chelating properties and enzyme blocking activity. Interest has been sustained since the symposium until the present day because lipoic acid has ideal properties for use as a drug.

(i) It possesses a very low toxicity (L_{D50} - in mice 275 mg /kg) with freedom from side effects at high doses.

(ii) It is not accumulated in the body. Lipoic acid has been shown to be rapidly absorbed and eliminated by mammals. After administration of almost toxic doses, the blood concentration returns to normal after 2 hours and in 6 hours excretion has been completed.

(a) An Antidote to Metal Poisoning

Reduzzi[62] in 1955 reported briefly that lipoic acid

chelated, in its dithiol form, to mercury and arsenic in the body. There have since been numerous reports of the use of lipoic acid as a chelating agent. Studies of acute heavy metal toxicity have shown RS-lipoic acid to be effective for the prevention and reversal of arsenic intoxication in mice[63-65], rats[64], rabbits[64] and dogs[66].

Grunert *et al.*[66] administrated 81 mg /kg of lipoic acid intraperitoneally to mice receiving a lethal dose of sodium arsenite (18 mg/kg) subcutaneously and reduced the mortality rate from 100% to 0%. Similar results have been obtained with animals poisoned with mercury. For example, Donatelli[67] intoxicated guinea pigs with 15 mg/kg mercurous chloride subcutaneously and treated them with 10 mg/kg of lipoic acid to obtain 60% recovery compared to no recovery with British Anti-Lewisite (BAL - the treatment for heavy metal poisoning). Lipoic acid is used to reverse completely the effects of copper intoxication (a symptom of Wilson's disease in humans), whereas BAL, penicillamine, and dithiocarbamate have been shown to be ineffective[68].

Beneficial effects of lipoic acid have also been reported for poisoning by nickel tetracarbonyl[69] and lead acetate[70] but tests have shown lipoic acid to be ineffective against the toxic effects of selenium(VII), uranium(VII) and nickel(II)[71,72].

(b) The Treatment of Liver Disease

In the treatment of liver disease, patients with any sort of liver disease suffer from extremely high levels of 2-oxo acids, pyruvic acid and lactic acid in the blood. Lipoic acid has been shown to decrease these levels in cases of hepatic coma[73], hepatitis[74], and cirrhosis[75] in human beings and in liver damage in mice induced by carbon tetrachloride[76] or paracetamol[77]. The pharmacological

activity of lipoic acid is thought to be due to its ability to catalyse the decarboxylation of 2-oxo acids activating pyruvic, 2-oxoglutarate, and lactic acid dehydrogenases[78]. Unfortunately the therapeutic use of lipoic acid in liver disease has not given consistent results. The numerous reports of the beneficial effects of lipoic acid against liver disease are countermanded by many instances of it having little or no effect at all. For example, whilst some patients have been roused out of hepatic[73] coma through treatment with lipoic acid, others have not responded at all[79].

Lipoic acid has been used very successfully in the treatment of poisoning by the mushroom *Amanita phalloides* which causes lesions of the liver. There are many reports of the successful treatment of this disease. Forty people in Czecho-Slovakia suffering the toxic effects of *Amanita phalloides* were treated with a prolonged infusion of up to 300 mg of lipoic acid per day and thirty nine lives were saved[80]. In 1970 Zaffri[81] cured seven cases of mushroom poisoning with high doses of lipoic acid. Recently, lipoic acid has been superseded in the treatment of mushroom poisoning by the more effective steroid, Prednisolone.

(c) Stability in Pharmaceuticals

Lipoic acid is stable in the solid form and can be easily used as a drug. When it is used in solution its light stability is increased by the addition of an equimolar amount of vitamin B_6[82].

(d) A Nutrient for Animals

When lipoic acid was found to play an essential role in the carbohydrate metabolism of living organisms attention was directed to its effect on animal nutrition. De Busk and Williams[83] reported appreciable increase in the growth rate and utilisation of food of chicks and rats when minute amounts of lipoic acid were incorporated in the diet. However, every attempt to achieve

stimulated growth in animals with lipoic acid as a nutrient have since failed[84-89].

1.14 BIOCHEMISTRY OF LIPOIC ACID

(a) 2-Oxo Oxidases

By far the most important physiological function of lipoic acid has been found to be its essential role in the oxidative decarboxylation of 2-oxo acids to form important thioesters of Co-enzyme A (Fig. 1.f). The enzymes responsible for these reactions are known as 2-oxo acid oxidases and work has shown them all to be structurally very similar[90,91]. It has been established that 2-oxo acid enzymes consist of a complex of three enzymes, one of which contains bound lipoic acid[92-98]. Detailed information has been obtained on the mechanism of oxidative decarboxylation of 2-oxo acids, by the investigation of model reactions. A generally accepted mechanism proposes that three enzyme mediated reactions participate:

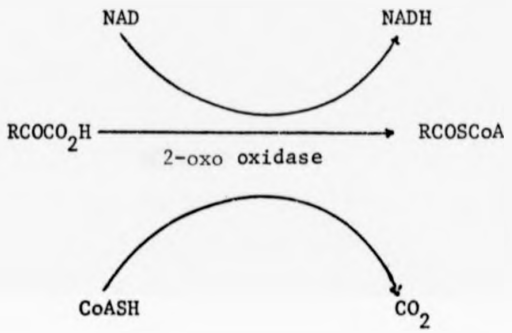
(i) Decarboxylation

The oxidative decarboxylation of 2-oxo acids is thought to involve[99,100] reaction of the acid with thiamine pyrophosphate to give an 'active aldehyde', or acylol (Fig. 1.g). A great deal of evidence has been found for this reaction which is catalysed by 2-oxo acid decarboxylase.

(ii) Acyl Transfer from Thiamine to Co-Enzyme A *via* Lipoic Acid

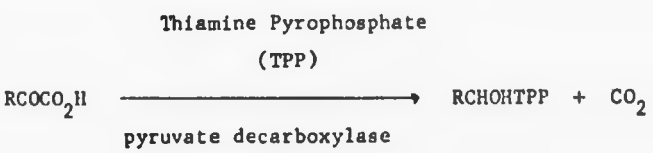
Gunsalus has shown that lipoic acid reacts with the acylol of thiamine pyrophosphate to form an addition complex which subsequently rearranges to form free thiamine and acylated lipoic acid[101,103,103] (Fig. 1.h). In this reaction, which is catalysed by lipoic acid reductase transacylase, the acylol moiety is oxidised to an acyl group and the lipoic acid is reduced. There is evidence to

FIG. 1.f



Oxidative Decarboxylation of 2-oxo Acids

FIG. 1.g



Formation of an Active Aldehyde in the
Oxidative Decarboxylation of 2-oxo Acids

FIG. 1.h

$$RCHOHTPP \xrightarrow[\text{Lipoic Acid Transacylase}]{\text{Lipoic Acid}} RCOS(CH_2)_2CHSH(CH_2)_4CO_2H$$

TPP = Thiamine pyrophosphate

Acyl Transfer from Thiamine to Lipoic Acid

FIG. 1.i

$$RCOS(CH_2)_2CHSH(CH_2)_4CO_2H \xrightarrow{\text{Co-Enzyme A}} AcCoA + HSCH_2CH_2CHSH(CH_2)_4CO_2H$$

Acyl Transfer from Lipoic Acid to Co-Enzyme A

indicate the acyl group is exclusively transferred to the C-6 sulphur atom. The reductase transacylase enzyme is then thought to transfer the acyl group from acylated lipoic acid to Co-enzyme A to give acylated Co-enzyme A and reduced lipoic acid (Fig. 1.i).

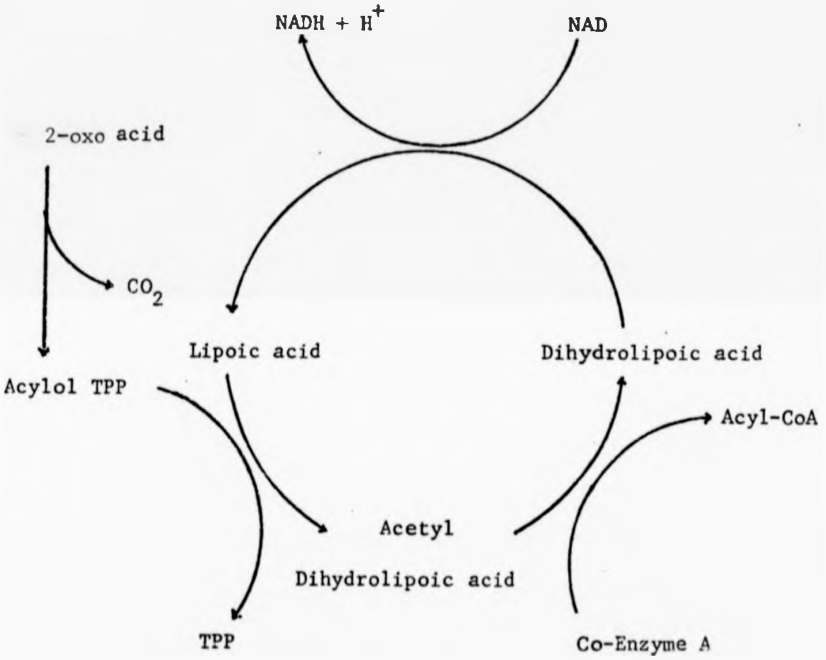
(iii) Oxidation of Dihydrolipoic Acid

The reduced form of lipoic acid is thought to be oxidised by the third enzyme of the 2-oxo acid oxidase complex, lipoamide oxidoreductase. In this way lipoic acid is regenerated to be used in further acyl transfer reactions and the oxidising agent, NAD^+ is reduced to NADH + H^+. An accepted model for the mechanism of this reaction capable of explaining all its observed chemical, physical and biochemical properties does not exist. However, several reaction schemes have been postulated by workers to explain their own results[104-107]. The overall conversion of 2-oxo acids to acylated Co-enzyme A is represented in (Scheme 1.9).

(b) Nature of Enzyme Complex and the Lipoic Acid Linkage in 2-oxo Acid Oxidases

Reed and Cox[92] in 1966 showed that the three enzymes of 2-oxo oxidase are linked by noncovalent bonds. These bonds were found not to exist between the decarboxylase and lipoamide oxidoreductase but each of the latter enzymes is bonded to lipoic acid transacylase. Lipoic acid reductasetransacetylase is the only enzyme of the 2-oxo oxidase complex containing protein-bound lipoic acid. According to Reed[108], lipoic acid is joined by a peptide link to the amino group of one of the lysine residues in the enzyme protein. Correspondingly, lipoamide and dihydrolipoamide are more effective than lipoic acid in model reactions[109,110], and N-RS-lipoyl-R-lysine is reduced three times as fast as RS-lipoamide by NADH and lipoamide oxidoreductase from *E. coli*.

SCHEME 1.9



Conversion of 2-oxo Acids to Acylated Co-Enzyme A

Recent work by Danson[111] indicates that the enzyme complex of pyruvate oxidase contains two types of lipoic acid residue. The form and purpose of the two residues is still being investigated but Frey[112] has postulated that one form catalyses the oxidative decarboxylation of pyruvate whilst the other has an, as yet, undefined electron transfer function. It is also possible that one type of lipoic acid residue has no biological function and is simply benign.

(c) The Importance of the Biological Role of Lipoic Acid

Two types of 2-oxo acid oxidases existing in living organisms have been thoroughly investigated. These are pyruvate oxidase and 2-oxoglutarate oxidase, both of which are involved in metabolic reactions essential to life itself.

Pyruvic acid, which is derived in animals from the breakdown of carbohydrates (respiration) or in plants by the assimilation of carbon dioxide and water using light energy (photosynthesis), undergoes oxidative decarboxylation with the enzyme pyruvate oxidase to produce a thioester, acetyl CoA. This thioester is of unique importance in biochemical processes since it can be further oxidised in a cycle of energy producing reactions to water and carbon dioxide. The reactions are known as the tricarboxylic acid cycle which is the major metabolic pathway of energy production in living organisms, and produces intermediates which are precursors to protein, porphyrins and nucleic acid. Acetyl Co-A also provides the C-2 unit which is the basic building block of all fatty acids in biological systems. Since fatty acids, upon oxidation, to CO_2 and H_2O, undergo a large negative change in free energy (e.g. for palmitic acid, $\Delta G = -233.8$ Kcal/mol) they offer a means of storing energy in living organisms. The high negative change is due to the oxidation of the highly reduced hydrocarbon radical attached to the carboxyl group of

the fatty acid to form a high energy bond in acetyl CoA which is then oxidised to CO_2 and water in the tricarboxylic acid cycle.

2-Oxoglutarate oxidase is equally as important as pyruvate oxidase because it is responsible for the oxidative decarboxylation of 2-oxo glutarate to succinyl CoA, which is one of the steps in the tricarboxylic acid cycle.

(d) Postulated Role in Photosynthesis

In 1952 Calvin and Barltrop[29] suggested the possible participation of lipoic acid in the conversion of light energy into chemical energy[114]. The proposal was based on the observation that plants, allowed to photosynthesise in the presence of $C^{14}O_2$, produced labelled intermediates of the tricarboxylic acid cycle rapidly in the dark but not at all during illumination. The only known way for these products to occur is *via* the lipoic acid-dependent transformation of pyruvic acid to acetyl CoA. Thus, Calvin and Massini[115] suggested that this process is blocked by light because most of the lipoic acid is maintained in the reduced state. A survey of 5; 6; and 7-membered cyclic disulphides[28,29] led to the further suggestion that a direct transfer of electrons from excited chlorophyll to lipoic acid occurs, resulting in dissociation of the disulphide linkage to give a dithiyl radical. The diradical was presumed to react with water to give an unstable thiol sulphenic acid which underwent subsequent transformation resulting in the formation of dihydrolipoic acid and molecular oxygen. This postulated sequence has been modified by Bradley and Calvin[113] in that lipoic acid is no longer assumed to be directly involved in the path of oxygen from water to molecular oxygen. Now, lipoic acid or a bound form of this substance is envisaged as participating in the electron transfer from chlorophyll to a pyridine nucleotide. The reduced pyridine nucleotide can then take part in reactions of the tricarboxylic acid cycle. Other investigations have produced

conflicting results[51,116], and there is no unequivocal evidence to support Calvin's hypothesis.

(e) Postulated Role in Oxidative Phosphorylation

The energy produced in organisms by the breakdown of carbohydrates and fatty acids is used to drive the phosphorylation of adenosine diphosphate (ADP) to the corresponding triphosphate (ATP). The terminal pyrophosphate bond is of very high energy and its hydrolysis to produce ADP yields a great deal of energy. The energy needed to drive metabolic reactions is always derived from the hydrolysis of ATP. The mechanism of this 'oxidative phosphorylation' process is not clearly understood but in 1976 Griffiths published a series of papers[117-120] which showed that lipoic acid may be involved. Lipoic acid was found to be present in high concentrations in the enzyme ATPase which catalyses the formation of ATP from ADP. This evidence together with the fact that 8-methyl lipoic acid inhibited oxidative phosphorylation suggested a specific but as yet unknown role for the lipoic acid in the process.

(f) Modified Lipoic Acids

Modified lipoic acids have been synthesised and their effect on the growth of *S. faecalis* 8043 has been noted. Analogues in which a CH_2 group in the chain between the carboxy group and the ring is replaced by an S atom[121] or an O-atom[122] are inactive as growth factors or cause a very weak growth effect on the bacteria. Substitution of a methyl group in the CH_2-chain produced an inhibitory effect[123], whereas introduction of an oxo group was found to give an inactive compound[124]. Substitution on the ring carbons was found to provide a growth inhibitory action. For example 7-methyllipoic acid , 7-hydroxylipoic acid[125], and 8-methyllipoic[126] acid all show inhibition of the growth of the bacteria. Alterations in the ring size or in the position of

the disulphide bridge led to loss of activity[127].

Much work has been done on the inhibition and substrate specificity of the enzyme lipoamide oxidoreductase. 2,4-Lipoic acid has been found unable to form an enzyme-substrate complex[127]. Replacement of C-3 of lipoic acid by S gave an analogue having no activity[121], whilst substitution of C-4 with O[122] gave a compound of much reduced affinity for the enzyme. The amide of this latter acid however gave almost the same activity as lipoamide[128].

Substitution on C-7 provided varying effects. 7-Methyllipoic acid was not oxidised by the enzyme but it had an inhibitory effect[6] while 7-hydroxylipoic acid was entirely inactive[115]. 7-Oxolipoic acid was oxidised more rapidly than the physiological substrate indicating an activating effect of the carbonyl group on the S-S bridge. Amides of lipoic acid were oxidised very quickly and bound tightly to the enzyme[11]. This suggests that the physiological form of the acid is an amide bound covalently to the enzyme.

1.15 CONCLUSION

It is perhaps because lipoic acid has been found in all organisms that considerable interest in its chemistry and biochemistry was generated. The structure of lipoic acid was quickly elucidated and between 1949 and 1965 a vast amount of research was carried out. However, as the major role of this acid was found to be the oxidative decarboxylation of 2-oxo acids and mechanisms were confirmed, research dwindled. Although there is still a great deal of interest in the pharmaceutical potential of lipoic acid and some curiosity about its possible role in photosynthesis, much information is still to be discovered. The absolute configuration has not been assigned with certainty. The role of lipoic acid in photosynthesis and

disease therapy remains unclear. Optical isomers of lipoic acid have been obtained by resolution only and not by a stereospecific route. The reaction of lipoic acid with nucleophiles has largely been ignored.

For a better understanding of the roles of lipoic acid in nature, more information about the compound is needed. The suggested function of lipoic acid in oxidative phosphorylation seems to have provided the impetus for a renewed effort.

CHAPTER 1 - REFERENCES

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CHAPTER 2

OBJECTIVES AND APPROACH TO THE WORK

2.1 OBJECTIVES

Only racemic lipoic acid is currently readily available and is the form frequently used in biological studies. This may complicate interpretation of biological results because the R-isomer is likely to interact differently in biochemical systems compared to the S-isomer. To facilitate interpretation of results, most pharmaceutical and biochemical studies require optically pure samples of both (R)- and (S)-lipoic acid. A demand for these enantiomers in high optical purity therefore now exists and encouraged us to undertake an investigation towards their synthesis. Our aim was to synthesise precursors and intermediates that would make possible quicker, easier, cheaper and higher yielding syntheses of (R)- and (S)-lipoic acid than those presently available. In the course of the work it was hoped that an optically active intermediate could be converted to a compound of known configuration enabling us to conclusively determine the absolute configuration of (R)- and (S)-lipoic acid.

8-Methyllipoic acid is a potent growth inhibitor[126] and therefore an antimetabolite of chemotherapeutic potential. As it is not commercially available and has not been made in optically pure forms, we have undertaken work on the synthesis of optical isomers of 8-methyllipoic acid.

2.2 CHOICES OF STRATEGY FOR THE SYNTHESIS OF OPTICAL ISOMERS OF LIPOIC ACID

There are three possible ways of synthesising a particular enantiomer of a compound with one chiral centre:

(i) Use of a suitable optically active starting material. The best source is a cheap, commercially available, naturally occurring compound. Optical activity is then carried through a synthetic sequence using reactions which either do not affect the asymmetric centre

or produce either complete retention or inversion of configuration.

(ii) Optical resolution of a racemic mixture derived either by non-enantioselective generation of an asymmetric centre at some stage in the synthesis or by use of a racemic starting material. The resolution is usually effected on an intermediate or end-product which contains an acid carboxyl group, or basic amino group, by formation of a salt with an optically active organic acid or base. The resulting diastereomers have different physical properties and are separable by fractional crystallisation.

(iii) Starting from an achiral precursor and using an optically active reagent for the enantioselective formation of one configuration rather than the other.

2.3 CHOOSING A STRATEGY

Intermediates in a number of syntheses of lipoic acid have been resolved. However, the biologically important isomer, the active R-enantiomer has been found difficult to obtain optically pure and has only ever been synthesised in overall yields approaching 1%[74,48]. The inactive S-enantiomer appears to be somewhat easier to separate from its antipode and has been obtained in about 15%[49] overall yield. Clearly optical resolution is not a good method for obtaining (R)- and (S)- lipoic acid in high yields and optical purities.

The generation of an asymmetric centre from an achiral precursor by means of an enantioselective reaction is an attractive possibility. However, there are certain disadvantages:

(i) The synthesis must be so designed to involve intermediates able to undergo stereoselective reactions. This means that the number of reactions and the type of intermediate used in the route is limited.

(ii) Difficulty is often experienced in obtaining 100% enantiomeric purity.

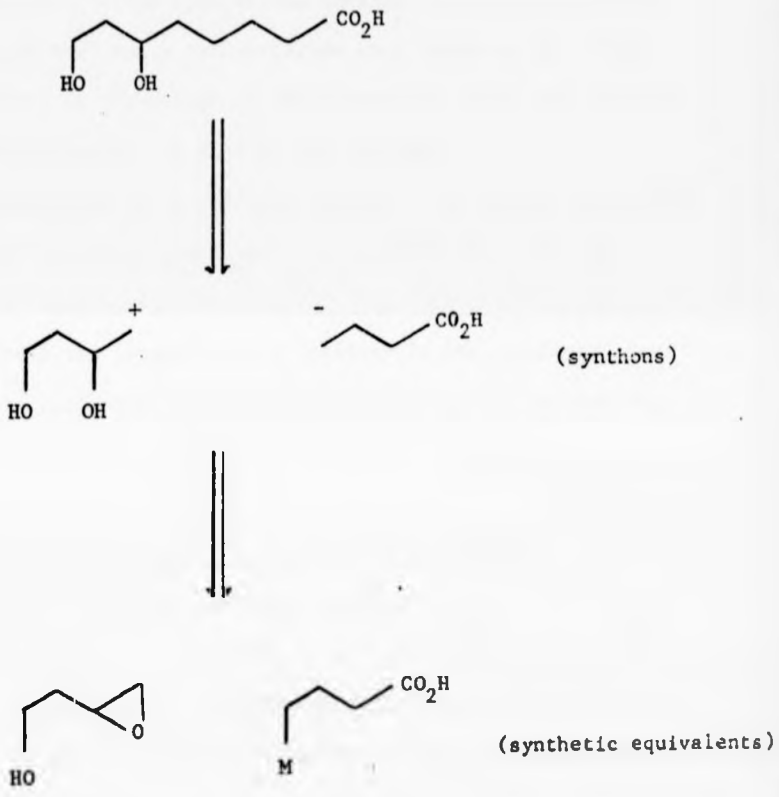
A more attractive idea seemed to be the use of cheap, readily available, optically active natural products. There is currently tremendous interest in the production of optically active blocks for use in enantiomer synthesis[129,130].

2.4 CHOSEN STRATEGY FOR THE SYNTHESIS OF (R)- AND (S)-LIPOIC ACID

Conception of organic syntheses for molecules of any complexity usually involves a stepwise procedure of working backwards from the structure of the final product to the structure of available starting materials[131,131a]. The routes for the synthesis of (R)- and (S)-lipoic acid were worked out in a similar manner.

As with all syntheses of lipoic acid, the precursor was envisaged as 6,8-dithiooctanoic acid. No problems were expected in obtaining this precursor in enantiomerically pure form, from optically active 6,8-dihydroxyoctanoic acid, because it is known that a hydroxyl group can be replaced by a thiol group with complete inversion of configuration[132]. 6,8-Dihydroxyoctanoic acid was to be derived from two four-carbon fragments, one of which would have the capability of forming a carbanion at one terminal carbon atom and a protected carboxylic acid group at the other. Since oxiranes are cleaved by nucleophiles regioselectively at the less hindered carbon atom and without affecting the configuration at the adjacent carbon atom, it was planned to join the fragments by reacting the carbanion centre with the terminal oxirane group of the other C_4 fragment (Scheme 2.1). This would generate a hydroxyl group with the desired configuration at the 6-position of the lipoic acid skeleton.

SCHEME 2.1



Retrosynthetic Analysis for the Synthesis of (R)- and (S)- Lipoic Acid

2.5 STEREOCHEMISTRY OF THE SYNTHESIS

In all the routes considered for the synthesis of optical isomers of lipoic acid, two changes of configuration are experienced:

(i) During the coupling of the two parts of the lipoic acid skeleton the terminal carbon atom of the oxirane undergoes a cleavage of a carbon-oxygen bond and a carbon-carbon bond replaces it. This alters the priority of the groups at the asymmetric centre and confers the opposite configuration, to that of the oxirane.

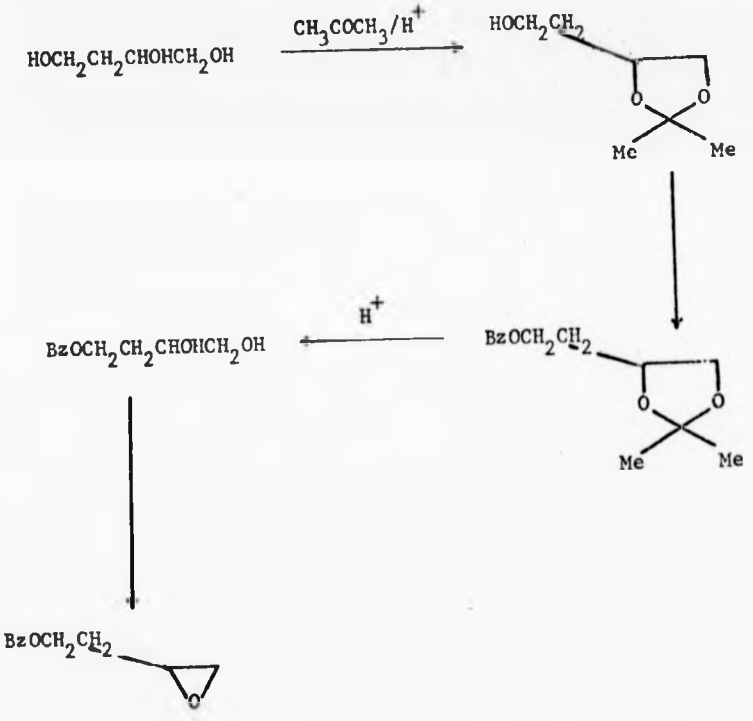
(ii) Substitution of the hydroxyl group of the various precursors of lipoic acid with a thiol group leads to complete inversion of configuration.

Because two inversions are involved in the syntheses, the configuration of lipoic acid will be the same as that of the starting material.

2.6 SYNTHESIS OF A SUITABLE OPTICALLY ACTIVE OXIRANE

The 1,2-oxirane containing C_4 fragment should also possess a protected oxygen function at C-4, which would become the C-8 sulphur bearing atom of lipoic acid. The oxirane is available from an optically active 1,2-diol by a conversion which has been carried out with 100% retention of configuration by a number of procedures[133-135]. The obvious starting material was butane-1,2,4-triol. Its vicinal hydroxyl groups could be blocked by an isopropylidene function allowing the remaining hydroxyl group to be benzylated. Hydrolysis of the ketal would give 4-benzyloxybutan-1,2-diol. (2-Benzyloxyethyl)oxirane derived from this diol (Scheme 2.2) would then be used for carbon-carbon bond formation, the benzyl protecting group being removed later in the synthesis. Butan-1,2,4-triol has been made in enantiomerically pure form from (S)-malic acid[136], a cheap naturally

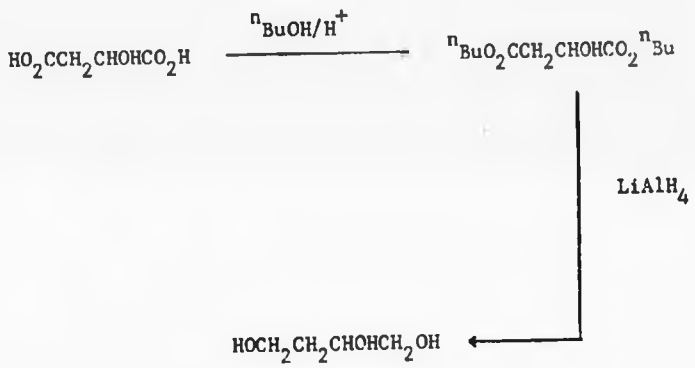
SCHEME 2.2



Synthesis of an Optically Active Oxirane from (S)-butane-1,2,4-triol

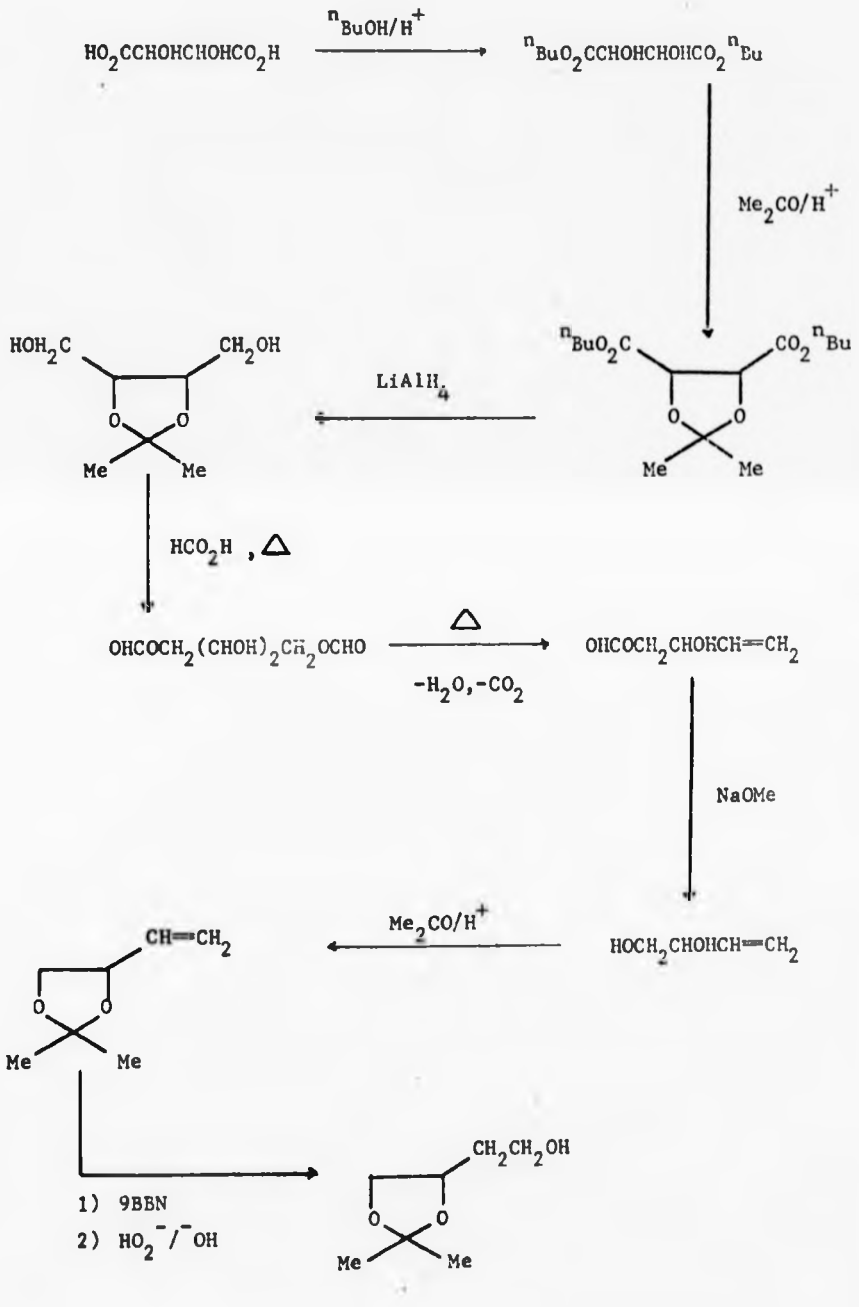
occurring compound[137], by esterification of the two carboxylic acid groups and their subsequent reduction to hydroxyl groups (Scheme 2.3). However, (S)-malic acid would give (S)-lipoic acid which is the unnatural and biologically less active isomer. To synthesise the highly active R-isomer of lipoic acid would require the use of (R)-malic acid which is thirteen times more expensive than its S-isomer and was not considered a good starting material. In order to be able to make both isomers of lipoic acid a cheap starting material of R-configuration was needed. Esters of (R,R)-tartaric acid were found to fulfill these stipulations[138] and work of Golding and Coates[139] provided a method of converting them to (R)-1,2-dihydroxybut-3-ene, a potential precursor of (R)-(2-benzyloxyethyl) oxirane. The hydroxy groups of the tartrate esters are protected and the two ester functions reduced to give (2S,3S)-isopropylidenethreitol. Hydrolysis to threitol, formation of a diformate, and its pyrolysis, resulted in cyclisation, carbene formation and elimination of carbon dioxide and water to give formates of 1,2-dihydroxybut-3-ene. The optical purity of the resulting product would have to be shown to be good before using this intermediate in the synthesis of optically pure lipoic acid. Conversion of (R)-1,2-dihydroxybut-3-ene to its isopropylidene derivative gives a material able to undergo regioselective hydroboration, generating a hydroxyl group exclusively at the terminal carbon atom. The resulting product, 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane is the enantiomer of an intermediate in the malic acid route to an optically active oxirane (Scheme 2.4). An advantage of tartaric acid as a starting material is that 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane can be prepared without the intermediate butane-1,2,4-triol, thus avoiding the practical problems encountered in the preparation of this

SCHEME 2.3



Synthesis of (S)-butane-1.2.4--triol
from (S)-Malic Acid

SCHEME 2.4



Proposed Route from Tartaric Acid to an Optically Active Intermediate of Enantiomers of Lipoic Acid

triol (due to high b.p., viscosity and water solubility).

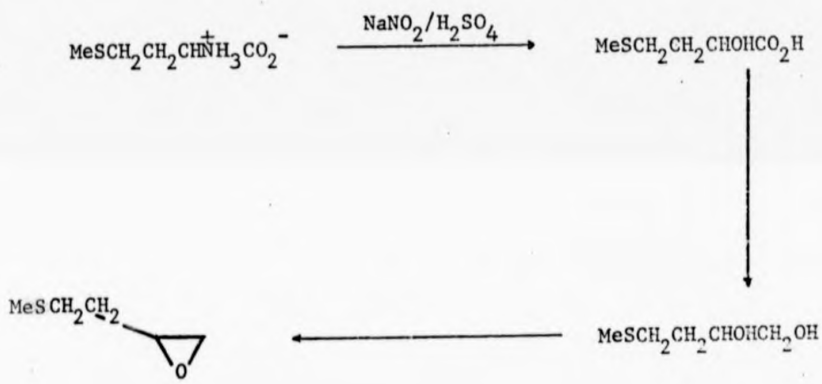
A second possible starting material for the synthesis of one enantiomer of lipoic acid is L-methionine. It possesses a four-carbon atom backbone, has an (S)-configuration in its natural levorotatory form and is very cheap. A simple route was conceived to a useful oxirane intermediate (Scheme 2.5). The first step was envisaged as deamination of methionine by formation of its diazonium salt and replacement by hydroxide ion. This particular reaction is known to occur with retention of configuration to give optically active (S)-2-hydroxy-4-methylthiobutanoic acid[140]. Reduction of the carboxylic group of this product with lithium aluminium hydride would be expected to yield (S)-4-methylthiobutane-1,2-diol, a compound easily convertible to the desired oxirane (S)-2-methylthioethyloxirane. The coupling of this optically active oxirane with the four-carbon atom carboxylic acid containing unit would lead to (S)-6-hydroxy-8-methylthiooctanoic acid. Conversion of the product to a dithiol and subsequent oxidation would lead to (S)-lipoic acid.

A slightly different approach was also considered whereby the optically active oxirane is converted to a thiirane. Nucleophilic attack of the carboxylic acid chain on this thiirane could give 8-methylthio-6-thiooctanoic acid.

2.7 THE NATURE OF THE CARBOXYLIC ACID GROUP

The concept of a 4-carbon fragment accommodating a carbanion at one terminal carbon atom and a carboxylic acid function at the other necessitates the use of a protecting group for the carboxyl. This is to stop unwanted intra-molecular reactions of the carbanion fragment and its precursor, the γ-halocarboxylic acid.

SCHEME 2.5



Synthesis of an Optically Active
Intermediate of Lipoic Acid from Enantiomers
of Methionine

A survey of the literature revealed no satisfactory means of masking a carboxy group. Orthoesters derived from *cis*-cyclohexane-1,3,5-triol, used for this purpose[141] are relatively inaccessible. Likewise, the masked carboxylic acids, 2-alkoxy-2-alkyl-(or aryl)-benzoxathioles, are tedious to prepare[142]. Orthoesters such as 1,1,1-triethoxyalkanes mask the carboxy group, but are too labile to hydrolysis for use as a protecting group[143]. Oxazolines have been recommended as masked carboxyl functions[144], but are rather difficult to hydrolyse and retain reactivity towards bases and nucleophiles. Perhaps the best masked carboxylic acids are 2-alkyl-2-methyl-1,3-dioxolanes[145-147]. Their hydrolysis yields an alkyl methyl ketone which can be transformed to a carboxylic acid by the haloform reaction. The major drawbacks are the relatively poor yields (40-50%)[148-150] in the haloform reaction.

It was apparent that there was a need for a superior carboxylic acid protecting group.

The fact that orthoesters are stable under basic conditions and undergo acidic hydrolysis makes the orthoester function a potentially useful protecting group. The fact that bicyclic orthoesters are much more stable than their acyclic analogues, as shown by Bouab *et al.*[151] has been put to good effect by using trioxa-adamantanes as masked carboxylic groups. However, because these compounds suffer the disadvantages, discussed above, 4-substituted-2,6,7-trioxabicyclo[2,2,2]octanes should be a better alternative, possessing sufficient stability for a wide application in organic synthesis whilst being available from 1,1,1-*tris*(hydroxymethyl)ethane, a very cheap readily available triol.

2.8 THE NATURE OF THE CARBANION

Grignard reagents are widely used in synthetic procedures and can be regarded as carbanion donors. Their ease of formation and known reaction with oxiranes made them the most obvious candidate for the combination of the two parts of the lipoic acid skeleton. This approach would require a halogen group at the terminal carbon atom of the masked carboxylic acid fragment. The second half of the lipoic acid skeleton was therefore envisaged as 4-halopropyl-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane. Synthesis of this compound would be possible from cheap 1-bromo-3-chloropropane[152] by reaction with the cyanide ion to produce 3-chlorobutyronitrile. Conversion of this product to 4-chloro-1,1,1-triethoxybutane could then be effected *via* its imino-ester. Transesterification with 1,1,1-*tris*(hydroxymethyl)ethane should result in the desired trioxabicyclooctane derivative.

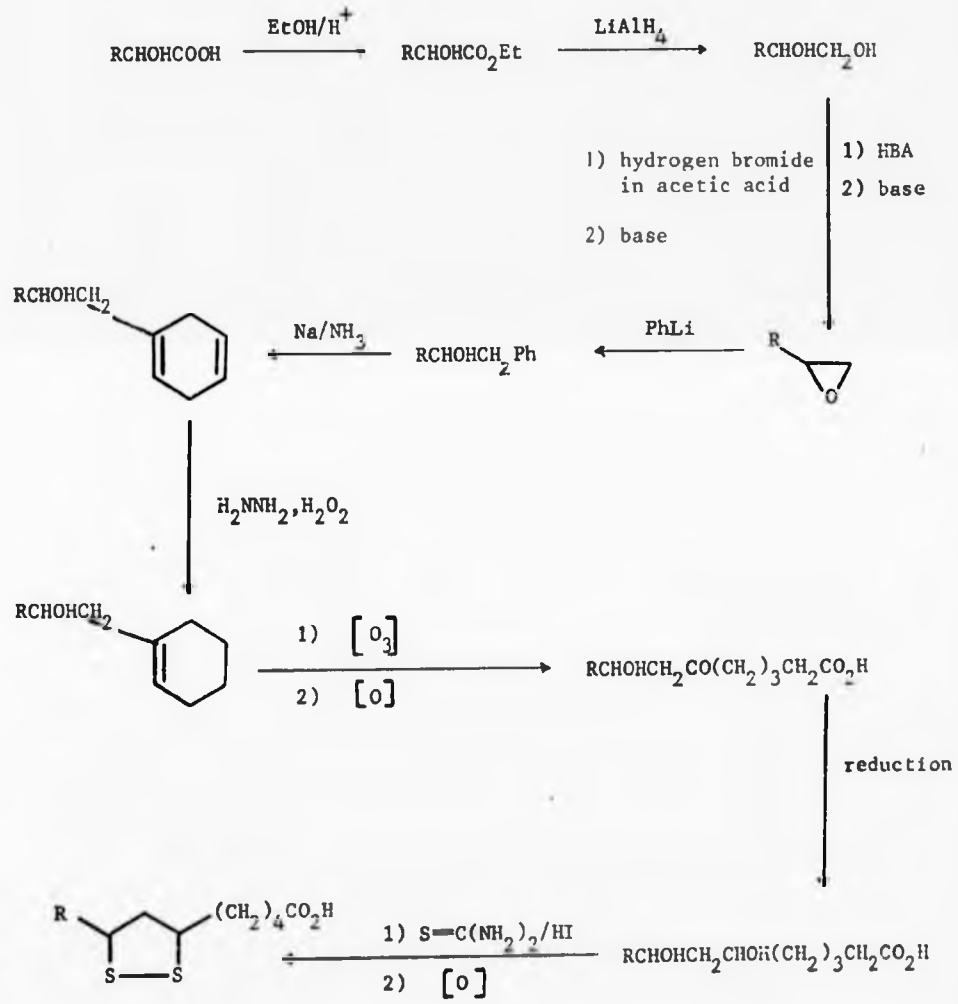
2.9 CARBON-CARBON BOND FORMATION

The coupling of two C_4-precursors of the lipoic acid molecule is the key step in the synthesis. The carboxyl of lipoic acid can then be released from its masked form. The O-benzyl protecting group can be easily hydrogenolysed to yield (R)-or (S)-6,8-dihydroxyoctanoic acid. The remainder of the synthesis of (R)- or (S)-lipoic acid is straightforward involving a literature procedure for the replacement of two hydroxyl groups by thiol groups.

2.10 CHOSEN STRATEGY FOR THE SYNTHESIS OF OPTICAL ISOMERS OF -8-METHYLLIPOIC ACID (Scheme 2.6)

A method of obtaining the nine carbon atom backbone of 8-methyllipoic acid with the necessary 6,8-functionality was suggested by the work of B. T. Golding *et al.*[133]. They reported an easy synthesis of optically active oxiranes from vicinal diols, in

SCHEME 2.6



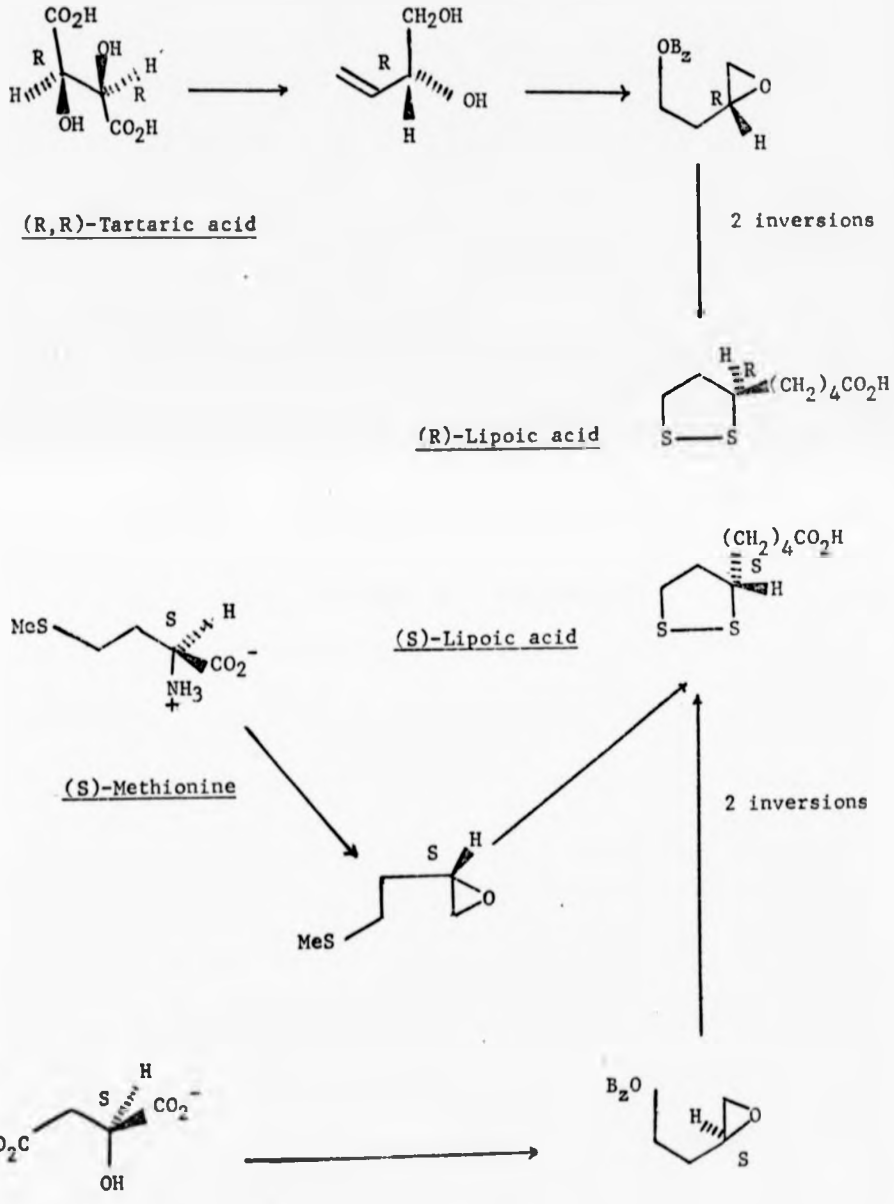
R = e.g. Me

A Possible Synthesis of optical isomers of 8-Alkyllipoic Acids from Lactic Acid and its Derivatives

particular (R)- and (S)-methyloxirane. Commercial (-)-(S)-ethyl lactate was reduced in high yield with lithium aluminium hydride to (+)-(S)-propane-1,2-diol. Reaction of the diol with hydrogen bromide in acetic acid was regioselective giving 94% of 2-acetoxy-1-bromopropane and 6% of 1-acetoxy-2-bromopropane. Treatment of the mixture of acetoxy-bromides with potassium pentylate gave 85% of analytically, and optically, pure (+)-(S)-methyloxirane distilled directly from the reaction mixture. As part of their aim to produce potentially useful optical active alcohols, Golding *et al.* reacted (+)-(S)-methyloxirane with phenyllithium and obtained (+)-(S)-1-phenylpropan-2-ol in 88% yield.

1-Phenylpropan-2-ol was recognised as a precursor to 8-methyllipoic acid. Birch reduction of this compound to 1-(2'-hydroxypropyl)cyclohexa-1,4-diene followed by regiospecific diimide reduction of the unsubstituted double bond to 1-(2'-hydroxypropyl)cyclohex-1-ene would result, after ozonolysis of the remaining double bond, in 8-hydroxy-6-oxononanaldehyde. Oxidation of the aldehyde function yields 8-hydroxy-6-oxononanoic acid. This acid would be expected to be enantiomerically pure since the configuration at carbon atom eight would have been carried through the synthesis unchanged from that of the starting oxirane. Stereoselective reduction of the keto group would introduce the second asymmetric centre of the lipoic acid analogue at carbon atom six (Scheme 2.6). A stereoselective reduction was thought to be possible by the use of sterically hindered reducing agents such as lithium aluminium dialkoxides Chapter 7 describes an investigation of a model system for this reduction. The remainder of the 8-methyllipoic acid synthesis involves straightforward, well-established reactions of previous lipoic acid syntheses.

SCHEME 2.7



Stereochemistry of the Proposed Methods of Synthesis of Optical Isomers of Lipoic Acid

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CHAPTER 3

THE PROTECTION OF CARBOXYLIC ACIDS AS ORTHOESTERS AND METHYL KETONES

A. THE SCOPE AND LIMITATIONS OF BICYCLIC ORTHOESTERS AS PROTECTING GROUPS

3.1 INTRODUCTION

The hydrate of a carboxylic acid, $RC(OH)_3$, is known as a carboxylic orthoacid. It is so thermodynamically unstable, compared to the ordinary carboxylic acid and water that its equilibrium concentration in aqueous solution is too small to be detectable[154]. Although orthoacids have never been isolated, their derivatives called orthoesters and consisting of three alkoxy groups each σ-bonded to the same carbon atom, are stable substances.

3.2 METHODS OF SYNTHESIS OF CARBOXYLIC ORTHOESTERS

In 1854, Williamson showed that chloroform reacts with sodium ethoxide and sodium amyloxide to form products now known to be triethyl and triamyl orthoformates[155,156].

$$CHCl_3 + 3NaOR \rightarrow HC(OR)_3 + 3NaCl$$

$$(R = C_2H_5,\ C_5H_{11})$$

The reaction of alkoxides and phenoxides with trihalomethyl compounds became a useful procedure for preparing trialkyl and triaryl orthoformates and triaryl orthobenzoates. However, the method did not find a wide application for orthoester synthesis because only a few trihalomethyl compounds such as chloroform, benzotrichloride and chloropicrin are suitable. These halocompounds lack β-hydrogen atoms and cannot therefore undergo base-promoted elimination reactions.

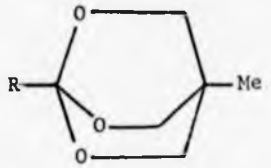
The most generally applicable synthesis of orthoesters involves alcoholysis of imino ester hydrochlorides, usually prepared by the addition of alcohol to a nitrile in the presence of hydrogen chloride. This preparation was first carried out by Pinner in 1883 when he made a series of trialkyl orthoformates from hydrogen cyanide[157,158]. It was not until twenty five years later that the Pinner synthesis was used as a general procedure for the preparation of orthoesters. In 1907 Reitter and Hess described the preparation of triethyl orthoacetate and triethyl orthopropionate[159]. Since that time many orthoesters have been made from nitriles and improved yields have been obtained with only slight modifications of the conditions of the original Pinner synthesis[160-162]. A vast amount of work has been carried out in recent years by McElvain and his co-workers, who used the Pinner reaction to prepare orthoesters as intermediates in the synthesis of ketene acetals.

Many orthoesters react with alcohols with exchange of alkoxy groups to form new orthoesters. This transesterification reaction results in an equilibrium which for acyclic products may often be displaced completely to one side by distilling a volatile product out of the reaction mixture. However, in some cases, a transesterified product is destabilised by steric factors and only mixed orthoesters can be isolated. The composition of the product mixture depends on reaction time, catalyst, catalyst concentration, efficiency of removal of product from the reaction mixture and the nature (particularly the size) of the alkoxy groups present. Thus, transesterification does not usually provide an efficient means of synthesising acyclic orthoesters due to severe steric limitations. The reaction is more difficult to achieve with secondary rather than primary alcohols and does not usually occur at all with tertiary alcohols. However, cyclic

orthoesters such as alkoxydioxolanes, alkoxydioxanes and more complex bicyclic and polycyclic orthoesters are readily formed by transesterification of acyclic orthoesters with diols, triols and polyols. The main driving force for this reaction is the very large increase in entropy. Many 1-alkyl-4-methyltrioxabicyclo[2,2,2]octanes have been made by transesterification of trialkyl orthoesters with 1,1,1-*tris*(hydroxymethyl)ethane (3a)[164-167].

3.3 PREPARATION OF 4-(3'-CHLOROPROPYL)-1-METHYL-2,6,7-TRIOXABICYCLO[2,2,2]OCTANE (3b)

As was discussed in Chapter 2, 2,6,7-trioxabicyclo[2,2,2]octanes should offer a number of advantages over present methods of carboxylic acid protection. Because of the potentially wide application of this method, much work was done in developing the idea, and as the orthoester (3b) was required for the synthesis of lipoic acid, its preparation became the first objective. A straightforward route using standard reactions was envisaged by a transesterification of 4-chloro-1,1,1-triethoxybutane (3c) with 1,1,1-*tris*(hydroxymethyl) ethane. The triethoxy derivative (3c) is available by alcoholysis of ethyl 4-chloroiminobutanoate hydrochloride (3d) which can be prepared from the commercially available 4-chlorobutyronitrile by the Pinner synthesis. Actually our synthesis of orthoester (3b) started from 1-bromo-3-chloropropane because it is over fifteen times cheaper than 4-chlorobutyronitrile to which it can be easily converted in good yield (treatment with KCN in refluxing aqueous ethanol). The synthesis of iminoester hydrochloride (3d) from 4-chlorobutyronitrile was achieved by adapting the general procedure of McElvain and Nelson[168]. However, instead of bubbling hydrogen chloride gas through dry ethanol until the correct weight of gas had been dissolved, addition of a calculated amount of acetyl chloride to ice-cooled dry ethanol, proved to



$MeC(CH_2OH)_3$

3a

3b R = $ClCH_2(CH_2)_2$

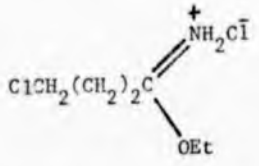
3e R = Me

3i R = $ICH_2(CH_2)_2$

$RC(OEt)_3$

3c R = $ClCH_2(CH_2)_2$

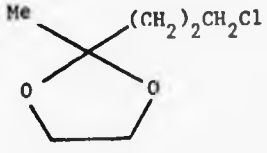
3f R = Me



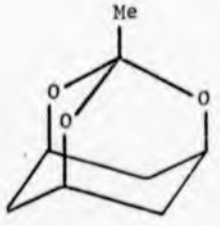
3d

$Cl(CH_2(CH_2)_2CO_2CH_2C(CH_2OH)_2CH_3$

3h



3j



3g

be a more convenient method. Generation of hydrogen chloride *in situ* did not adversely affect the yield of iminoester. A possible competing side reaction in the synthesis of iminoester hydrochlorides from nitriles is the decomposition of the imidate salt to an amide and an alkyl chloride:

$$RC(=NH_2^+)OR'Cl^- \rightarrow RCONH_2 + R'Cl$$

Neither of these products were observed in the preparation of the iminoester (3d) by keeping the reaction mixture at no more than 0^o for the entire preparation.

When the alcoholysis of iminoester (3d) was carried out in refluxing ether/ethanol, the conditions employed by McElvain and Nelson[168], 4-chloro-1,1,1-triethoxybutane (3c) and ethyl 4-chlorobutanoate were obtained in approximately equal amounts. The amount of normal ester by-product was drastically reduced by using conditions used for the very first syntheses of orthoesters. For example, when the iminoester (3d) was dissolved in ethanol and left at room temperature for 2 days, the desired product was obtained, containing only five per cent of the ester impurity. The contaminating compound was found to co-distil with the product but it was effectively removed by phase-transfer catalysed hydrolysis with sodium hydroxide, to the acid, which was simultaneously extracted into the aqueous phase leaving pure orthoester (3c) in the organic layer. The yield of pure product obtained in this way was comparable to those claimed by McElvain and Nelson for straight chain orthoesters. The highest yields were obtained when the iminoester had been pumped at low pressure, overnight, to ensure complete removal of hydrogen chloride. It has been shown[169] that the appearance of normal ester during the alcoholysis of imidate salts under anhydrous conditions is due to the acid-catalysed decomposition of the orthoester as it is formed, by reaction with the acidic unreacted imino-

ester hydrochloride. This reaction is impossible to inhibit during the course of the alcoholysis and the decomposition of a small amount of orthoester must be accepted.

Acyclic orthoesters are more reactive toward acid hydrolysis than almost any other class of organic compounds. For this reason orthoester (3c) had to be stored under anhydrous conditions with potassium carbonate, otherwise there was contamination with hydrolysis products. Orthoester (3c) was reacted with an equimolar amount of triol (3a) in benzene with overnight reflux to yield 66% of pure orthoester (3b).

The overall yield for the preparation of orthoester (3b) from 1-bromo-3-chloropropane was 16% and meant that the reasonably large amount of orthoester needed for experiments was obtainable.

3.4(a) KINETICS OF HYDROLYSIS OF SOME ORTHOESTERS

In order to assess the potential of 2,6,7-trioxabicyclo[2,2,2] octanes as protecting groups of wide applicability, some of their chemical and physical properties had to be studied. It is essential to show that the group is stable enough to survive an organic preparation intact and also to ensure that the free acid can be liberated easily under mild conditions.

An indication as to the stability of the 2,6,7-trioxabicyclo-[2,2,2]octane group can be obtained by a comparison of its rate of hydrolysis in mildly acidic conditions with those of other orthoesters. In order to broadly examine substituent effects, the hydrolysis of 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane (3e) was compared with that of the bicyclo octane (3b). Comparison of the rates of hydrolysis of 2,6,7-trioxabicyclo[2,2,2]octanes with those for the triethoxy analogues would be informative because the latter are known

to be too reactive to be useful in syntheses as protecting groups. Likewise comparison with the rate of hydrolysis of a 2,8, 9-trioxa-adamantane derivative would be useful as such functional groups have been used in several organic preparations which it survived. The experiments should also show that the 2,6,7-trioxabicyclo[2,2,2]octane group is sufficiently reactive towards acidic hydrolysis to enable the carboxy group to be released under mild conditions.

(b) RESULTS AND DISCUSSION

1-Methyl-2,8, 9-trioxa-adamantane was prepared by reacting 1,1,1-triethoxyethane (3f) with 1,3,5-cyclohexanetriol with Lewis acid catalysis by boron trifluoride diethyl etherate. The triethoxy compound was made from acetonitrile by the same method as that used for the preparation of 4-chloro-1,1,1-triethoxybutane from 4-chlorobutyronitrile. Orthoester (3f) was also reacted with 1,1,1-*tris*(hydroxymethyl)ethane to give a good yield of 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane. Orthoesters (3b and 3c) were prepared as discussed previously. Hydrolysis of all five orthoesters were carried out under identical conditions at 30° in water-dioxan (2:3) at a pH of 4.66 buffered with acetic acid and sodium acetate. A constant ionic strength was maintained by the presence of a calculated amount of sodium chloride. The reactions were monitored by following the appearance of u.v. absorption from the diol ester product (λ_{max} 225 nm). The results are given in Table 3.1

The rates of acidic hydrolysis of the various orthoesters gave an order of reactivity of 3f > 3c >> 3e > 3b >>> 3g. The half lives of these compounds indicate the following.

(i) The diminished reactivity of the bicyclic orthoester over the

TABLE 3.1

Hydrolysis of Cyclic and Acyclic Orthoesters in Water-Dioxan (2:3) with Acetic Acid-Sodium Acetate Buffer (pH 4.66) of Constant Ionic Strength at 30°

Orthoester	k/secs^{-1}	Standard Mean Deviation	$t_{\frac{1}{2}}$/sec	Standard Mean Deviation
1,1,1-Triethoxyethane (3f)	1.434×10^{-2}	3.629×10^{-5}	48.326	1.663×10^{-3}
1,1,1-Triethoxy-4-chlorobutane (3c)	2.606×10^{-3}	2.348×10^{-6}	3.360×10^{2}	2.642×10^{-3}
1,4-Dimethyl-2,6,7-trioxa-bicyclo[2,2,2]octane (3e)	3.456×10^{-4}	5.510×10^{-7}	2.005×10^{3}	1.976×10^{-3}
4(3'-Chloropropyl)-1-methyl trioxa-2,6,7-bicyclo[2,2,2]octane (3b)	1.078×10^{-4}	3.818×10^{-7}	6.428×10^{3}	5.152×10^{-3}
1-Methyl-2,8,9-trioxa-adamantane (3g)	-	-	-	-

acyclic ones, probably due mainly to the more positive entropy of activation of the latter.

(ii) That 1,1,1-triethoxyalkanes are too labile to be of use as a masked carboxylic acid.

(iii) That the trioxa-adamantane derivative is stable to mild acid and requires more severe conditions, for hydrolysis, than those needed for the 2,6,7-trioxabicyclo[2,2,2]octane group.

(iv) That changing the alkyl group does not alter the large difference in rates of hydrolysis between acyclic and bicyclic orthoesters.

(v) That the 2,6,7-trioxabicyclo[2,2,2]octane group possesses suitable activity for applications in synthesis as a protecting group.

(c) POSSIBLE MECHANISMS OF ORTHOESTER HYDROLYSIS

The fact that trialkyl orthoformates and acetates are extremely sensitive to acid-catalysed hydrolysis, and that the products are neutral and relatively stable, has meant that much work has been done on the hydrolysis of these compounds. The first examples of general acid catalysis to be discovered were hydrolysis reactions of orthoesters. Most of the vast amount of evidence concerning hydronium ion-catalysed orthoester hydrolysis is consistent with an A 1 mechanism (Scheme 3.1) involving rate-limiting formation of the dialkoxy carbonium ion. This is not surprising since the dialkoxy carbonium ion is a very stabilised cation and its conversion to hydrolysis products will therefore be faster than its formation. Carbonium ion formation may involve either rate-limiting proton transfer from the acid catalyst to the orthoester or it may involve rate-

SCHEME 3.1

$$RC(OR')_3 + HA \xrightarrow{slow} R\overset{+}{C}(OR')_2 + R'OH + A^-$$

$$R\overset{+}{C}(OR)_2 + H_2O \xrightarrow{fast} RCO_2R' + R'OH + H^+$$

A1 mechanism of orthoester hydrolysis with carbonium ion as the rate limiting step

SCHEME 3.2

(1) $RC(OR')_3 + HA \xrightarrow{slow} RC(OR')_2 \xrightarrow{fast} R\overset{+}{C}(OR')_2 + R'OH$

(2) $RC(OR')_3 + HA \xrightarrow{fast} RC(OR')_2 \xrightarrow{slow} R\overset{+}{C}(OR')_2 + R'OH$

Two S_E2-mechanisms for the formation of the carbonium ion in an A1 mechanism of orthoester hydrolysis

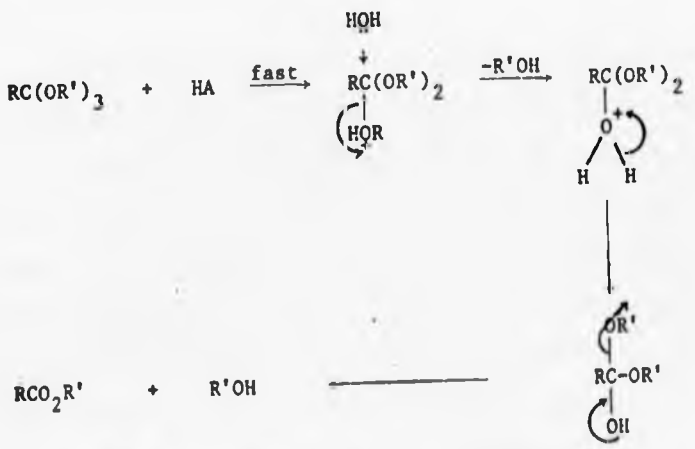
SCHEME 3.3

$$RC(OR')_3 + HA \xrightarrow{fast} R\overset{+}{C}(OR')_2 + R'OH + A^-$$

$$R\overset{+}{C}(OR')_2 + H_2O \xrightarrow{slow} RCO_2R' + R'OH + H^+$$

A1 mechanism of orthoester hydrolysis with addition of water as the rate limiting step

SCHEME 3.4



An A2 mechanism of orthoester hydrolysis

limiting carbon-oxygen bond cleavage in a hydrogen bonded complex of the acid and the orthoester. Both these processes are examples of bimolecular electrophilic displacements (S_E 2) of dialkoxy carbonium ions from alkoxy oxygen (Scheme 3.2).

Bouab *et al.* [151], however, produced evidence that suggests the hydrolysis of 2,8,9-trioxa-adamantane, at least, occurs with addition of water to the carbonium ion as the rate-determining step (see Scheme 3.3). The 2,8,9-trioxa-adamantane was found to have a large negative entropy of activation compared to the large positive entropy of acyclic orthoester hydrolysis. The large negative entropy implies a bimolecular reaction involving either direct substitution (Scheme 3.4) i.e. an A2 mechanism, or rate determining attack by water on a preformed carbonium ion. The possibility of an A2 mechanism was ruled out, since if this were the case, 2,8,9-trioxabicyclo[3,3,1]nonane, whose structure and lone pair orientation in the ground state is similar to those of the trioxa-adamantanes, would undergo hydrolysis by the same mechanism at a similar rate. It does not, it reacts very much faster, at a rate comparable with acyclic compounds.

(d) CONCLUSION

Our kinetic experiments showed that whilst acyclic orthoesters were rapidly hydrolysed under mildly acidic conditions, the adamantane derivative showed no measurable hydrolysis thus supporting Bouab's view that hydrolysis of the two types of compound occurs by different mechanisms. The fact that the bicyclic orthoesters also underwent hydrolysis suggests that a similar mechanism as the A1 mechanism, thought to occur for the hydrolysis of acyclic orthoesters, is operating. However, this cannot be conclusively proved without

measuring the entropy of activation of hydrolysis of bicyclic orthoesters. It is not surprising that the mechanism of hydrolysis of adamantanes and bicyclic orthoesters may be different since once the carbonium ion is formed and the compounds adopt the favourable chair configuration, the oxygen anion and carbonium ion will be held closely together in the adamantane but apart in the bicyclic orthoester (Fig. 3.a). Thus attack of water will be rapid on the stable and easily approached carbonium ion of the bicyclic orthoester, carbonium ion formation becoming the rate determining step. However, as the positive and negative charges of the adamantane hydrolysis intermediate are held closely together, formation of the starting molecule will be favoured, pulling the equilibrium over to favour starting material, and the addition of water thus becomes the rate determining step.

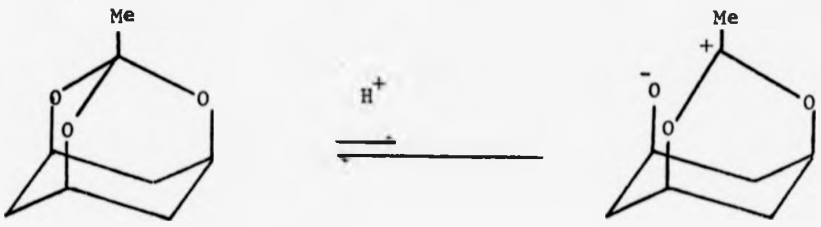
The fact that for both the 1,1,1-triethoxyalkanes and the 4-alkyl-1-methyl-2,6,7-trioxabicyclo[2,2,2]octanes, the 4-methyl derivative was less stable than the corresponding 4-(3'-chloropropyl)-substituted compound indicates the influence of the 3-carbon atoms of the propyl chain in destabilising the carbonium ion of the hydrolysis intermediate.

Whilst no conclusive evidence concerning the mechanism of bicyclic orthoester hydrolysis has been obtained, the results that have been acquired indicate that whilst bicyclic orthoester derivatives behave in a similar way to their acyclic analogues they possess a significantly greater stability which should enable them to be easily handled and capable of surviving synthetic procedures carried out under basic or perhaps even neutral conditions.

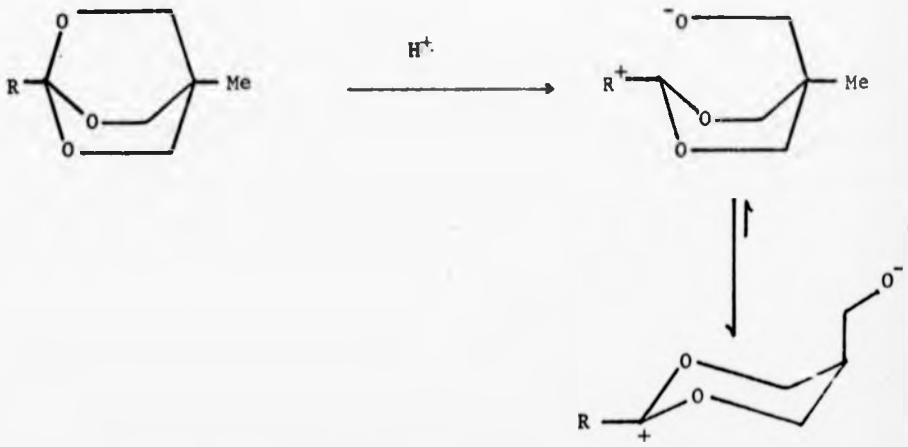
3.5 PREPARATION OF 2',2'-*bis*(HYDROXYMETHYL)PROPYL 4-CHLOROBUTANOATE

Experiments were carried out to optimise the conditions for hydrolysis of the 2,6,7-trioxabicyclo[2,2,2]octane group to 2',2'-*bis*(hydroxymethyl)-propyl 4-chlorobutanoate

FIG. 3.a



Intermediate in the Hydrolysis of
1-Methyl-2,8, 9-trioxa-adamantane
with Carbonium Ion and Oxygen Anion
Held Closely Together in the Favourable
Chair Conformation



Intermediate in the Hydrolysis of
4-Alkyl-1-methyl-2,6,7-trioxabicyclo 2,2,2 octanes
with Carbonium Ion and Oxygen Anion Held Apart
in the Favourable Chair Conformation

so that a general procedure could be established for all orthoesters of this type. The pure diol ester obtained in this way could then be used to examine ways of releasing the free acid group. All experiments were conducted on orthoester (3b).

Orthoester (3b) was found to undergo complete hydrolysis in a matter of minutes under very mild acidic conditions (0.05 M hydrochloric acid), to give pure 2',2'-*bis*(hydroxymethyl)propyl 4-chlorobutanoate (3h). However, the orthoester (3b) was found to be relatively stable in neutral aqueous solution, undergoing less than 10% hydrolysis at room temperature overnight.

It is interesting to note that the diol ester (3h) showed a similar instability as 1,1,1-*tris*(hydroxymethyl)ethane monoacetate which undergoes intermolecular transesterification on storage (see below).

3.6 PREPARATION OF THE FREE ACID FROM 2',2'-*bis*(HYDROXYMETHYL)PROPYL 4 -CHLOROBUTANOATE

Free acid can be obtained from diol ester (3h) using conditions for normal ester hydrolysis, i.e. by treatment with either strong acid or mild base. Obviously, use of strong acid is the less desirable method, but still of use in certain circumstances.

When diol ester (3h) was stirred with aqueous THF containing sodium hydroxide (0.5 M soln) for 3 hours at room temperature γ-butyrolactone was obtained as the only product. The 4-chlorobutanoic acid resulting from hydrolysis of the diol ester cyclises to the lactone, which was obtained in a pure state and in a yield of 82%. This experiment indicated the conditions needed to obtain free acid from other diol esters. Doering and Levy[165] observed that on warming 1-methyl-2,6,7-trioxabicyclo[2,2,2]octane to 100° for 30 mins. and evaporating to dryness, 1,1,1-*tris*(hydroxymethyl)methane was

recovered.

We found that overnight reflux of bicyclic orthoester (3b) with water produced pure γ-butyrolactone and triol. This intriguing hydrolysis under neutral conditions was then attempted with 1-methyl-2,8, 9-trioxa-adamantane, giving pure acetic acid and cyclohexane-1,3,5-triol. This was a surprising result because in cases where the trioxa-adamantane had been used as a protecting group refluxing with strong acid[170] was used for liberation of the carboxylic acid.

More severe conditions were needed to achieve hydrolysis under acidic conditions, γ-butyrolactone again being obtained, when bicyclic orthoester (3b) was refluxed with 1 M hydrochloric acid overnight.

In the search for a very mild procedure for the conversion of bicyclic orthoester (3b) to carboxylic acid, methods for transesterifying (3b) were investigated. When (3b) in excess dry ethanol containing 0.1 M hydrochloric acid was kept for 10 mins. at room temperature, a small amount of 4-chloro-1,1,1-triethoxybutane was formed. Longer reaction times did not increase the amount of this compound. Refluxing (3b) overnight in wet ethanol or methanol containing 0.1 M hydrochloric acid gave the corresponding 4-chlorobutanoate ester in good yield. If 2,2,2-trichloroethanol were used in this type of reaction, the resulting 2,2,2-trichloroethyl ester could be subsequently cleaved under very mild conditions (zinc in dimethyl formamide). Lack of time prevented exploration of this idea.

3.7 AN APPLICATION OF 2,6,7-TRIOXABICYCLO[2,2,2]OCTANES IN THE SYNTHESIS OF ALKYLCOBALOXIMES CONTAINING ESTER AND CARBOXY GROUPS

Alkylcobaloximes and cobalamins are usually prepared by reacting a cobalt(I) complex consisting of a tetradentate ligand,

bromine atom and base ligand (H_2O, pyridine, etc.) with an alkyl halide. Ester or carboxy functions in the σ-alkyl groups of these compounds are required for studies of adenosylcobalamin-dependent enzyme reactions[171].

Alkyl iodides are generally used in the preparation of alkylcobaloximes from Co^I compounds. Thus, the chloro function at orthoester (3b) was exchanged for an iodo group. When orthoester (3b) was treated with sodium iodide (10 mole equivalents) in dry acetone at room temperature it was converted to diol ester (3h). Ring opening of the trioxabicyclooctane system is probably caused by acidic species in the reaction mixture, and so the reaction of (3b) with sodium iodide was repeated in the presence of sodium bicarbonate. After two days at room temperature, work-up afforded a 65% yield of pure iodo derivative (3i).

A sample of 4-(3'-iodopropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (3i) was given to M. P. Atkins and P. J. Sellars* who successfully used it to synthesise 4-[*bis*-(dimethylglyoxime)(pyridine)cobalt]butanoic acid. The carboxyl group of 4-iodobutanoic acid was masked as a 2,6,7-trioxabicyclo[2,2,2]octane to overcome the problem of lactonisation of the halogeno-acid, under the basic reaction conditions used to prepare alkylcobaloximes, and to make product isolation easier. Bicyclic orthoester (3i) was treated with 1 mol equivalent of (pyridine)cobaloxime(I) (prepared by reducing bromo(pyridine)cobaloxime with $NaBH_4$ in ethanol) for 16 h/r.t. to give the desired product(a) (Scheme 3.5).

Cobaloxime (a) was treated with 0.5 M hydrochloric acid (30 mins. at room temperature) to give the diol ester(b)(Scheme 3.5). Methanolysis of this compound by sodium methoxide in methanol gave the methyl ester (c) (Scheme 3.5) in 78% overall yield from(a). Hydrolysis of(a) with a

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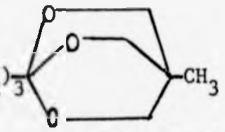
*Department of Chemistry and Molecular Sciences
University of Warwick, Coventry, CV4 7AL

SCHEME 3.5

$BrCo^{I}(dmgH)_2py \xrightarrow{RI} RCo(dmgH)_2py$

(a)  $R = (CH_2)_3$ ... CH_3

(b) $R = (CH_2)_3CO_2CH_2CMe(CH_2OH)_2$

(c) $R = (CH_2)_3CO_2Me$

(d) $R = (CH_2)_3CO_2H$

The synthesis of alkylcobaloximes containing ester and carboxy groups using 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo [2,2,2]octane

two-phase system (CH_2Cl_2-aqueous KOH) gave, after neutralisation of the aqueous layer and addition of pyridine, 62% of the acid(d)(Scheme 3.5).

3.8 VIABILITY OF 2,6,7-TRIOXABICYCLO[2,2,2]OCTANES AS PROTECTING GROUPS

The information imparted in the previous section shows that 2,6,7-trioxabicyclo[2,2,2]octanes are masked carboxylic acids from which the free acid is easily liberated. The synthesis of the various 2,6,7-trioxabicyclo[2,2,2]octanes by transesterification of the triethoxy precursor was a viable means for obtaining samples of various derivatives so that their properties could be investigated. The method was also successfully used to obtain a large amount of orthoester (3b) which was needed for the synthesis of lipoic acid. However, it is not a suitable general procedure for the synthesis of many other trioxabicyclo[2,2,2]octanes. Reasons for this are two-fold. Firstly, iminoestersalts upon alcoholysis, even with the exclusion of moisture and hydrogen chloride from the reaction mixture, often form carboxamide, alkyl chloride, carboxylate ester, and dialkyl ether by-products. The reactions leading to these by-products compete with the formation of orthoester to such an extent that little or no desired product may be obtained. The nature of the competition between amide, ester and orthoester formation, is determined largely by the structure of the iminoester hydrochloride, which clearly imposes limitations on the types of trioxabicyclooctane available by this synthesis. Thus, the conditions for the syntheses of trioxabicyclooctanes will have to be determined and optimised for each individual case. Secondly, the method of synthesising trioxabicyclooctanes from nitriles involves three steps in an overall yield of less than 20%.

For the trioxabicyclooctane to be a useful protecting group it should be made from any type of carboxylic acid in a simple one-step reaction with good yield. Direct esterification offers this possibility.

3.9 BICYCLIC ORTHOESTERS BY DIRECT ESTERIFICATION

Little work has been done on the synthesis of any type of bicyclic orthoester by direct esterification of an acid. Barnes *et al.*[172] have shown that stronger organic acids such as di- and tri-, chloro- or fluoro-acids do react directly with 1,1,1-*tris*(hydroxymethyl)ethane. However, no yield above 51% was obtained and other carboxylic acids such as caproic acid failed to form the bicyclic orthoester. Although this work was discouraging, another report[173] claimed 80% yields and more for the synthesis of a wide variety of dialkyl-2,6,7-trioxabicyclo[2,2,2] octanes, by direct reaction of the acid with 1,1,1-*tris*(hydroxymethyl)ethane. This method consisted of heating at reflux a mixture of 1,1,1-*tris*(hydroxymethyl)ethane and a carboxylic acid, either neat or in a solvent, with acid catalyst. The reaction was performed in a flask fitted with an 8-tray Oldershaw column and variable take-off. By adjusting the rate of collection of distillate, it was possible to strip off the solvent and then distil over the bicyclic orthoester product. The use of an Oldershaw column, a sophisticated and extremely expensive piece of apparatus, means that this method is not suitable as a general procedure for the synthesis of the bicyclic orthoester.

1,4-Dimethyl-2,6,7-trioxabicyclo[2,2,2]octane is a stable, white crystalline solid, easily obtained by sublimation, and so the formation of this particular compound, from acetic acid was investigated.

Preliminary experiments were conducted, by refluxing

1,1,1-*tris*(hydroxymethyl)ethane and acetic acid with p-toluenesulphonic acid. Molecular sieves were added to the reaction mixture to remove water formed during the reaction. However, use of a high boiling ether solvent, in which the triol was suspended, or dimethyl sulphoxide which dissolved the triol, gave no trioxabicyclooctane (t.l.c. monitoring). When the reaction was carried out in benzene, and water was removed by a Dean and Stark trap, half the theoretical amount of water for formation of the trioxabicyclooctane was obtained. However, the ^{1}H n.m.r. spectrum of the crude product showed it to consist of a mixture of the mono- and diacetates of 1,1,1-*tris*(hydroxymethyl)ethane in a ratio of 2:1. Prolonged heating of this mixture with a catalytic amount of p-toluenesulphonic acid at 120° and 0.1 mmHg in a Kugelröhr apparatus gave a small amount of the desired trioxabicyclooctane which sublimed from the reaction mixture. Although this product was pure, it was obtained in a yield of only 10%.

In principle, the monoacetate of 1,1,1-*tris*(hydroxymethyl) ethane can be dehydrated to 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane. To simplify examination of this possibility it was decided to work with the pure monoacetate rather than the mixture with diacetate obtained from heating acetic acid with 1,1,1-*tris*(hydroxymethyl)ethane.

1,1,1-*Tris*(hydroxymethyl)ethane monoacetate was synthesised as follows: 1,1,1-*tris*(hydroxymethyl)ethane was stirred in acetone containing concentrated sulphuric acid to form 5-hydroxymethyl-2,2-dimethyl-5-methyl-1,3-dioxane; the free hydroxy group of this compound was acetylated by refluxing it with acetic anhydride and pyridine; hydrolysis of the ketal afforded the desired product. It was heated in a Kugelröhr apparatus at 120° and 0.1 mmHg with a catalytic amount of p-toluenesulphonic acid. Pure 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2] octane sublimed out of the mixture in 6% yield. In an attempt to increase

this yield, 1,1,1-*tris*(hydroxymethyl)ethane monoacetate was refluxed in benzene containing p-toluenesulphonic acid, with azeotropic removal of water. A mixture of mono- and diacetylated triol in ratio 2:1 was obtained. Use of the monoacetyl triol had, therefore, proved no better than the methods using acetic acid directly and indeed heating the neat oil at 120° and 0.1 mmHg with p-toluenesulphonic acid in a Kugelröhr apparatus resulted again in a 10% yield of pure 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane.

Heating acetic acid with 1,1,1-*tris*(hydroxymethyl)ethane or heating the monoacetate of the triol probably causes equilibration of triol with its monoacetate, diacetate and possibly triacetate. From the resulting mixtures a small yield of trioxabicyclooctane can be sublimed. 1,1,1-*Tris*(hydroxymethyl)ethane monoacetate decomposed over 3 days at room temperature or over a few weeks at 0°C, to a mixture of triol, and mono- and diacetate. These reactions probably involve intermolecular transesterifications.

In all attempts to form the trioxabicyclooctane from either acetic acid and triol (3a) or from triol monoacetate two competing reactions have been encountered: cyclisation of the monoacetate to the desired trioxabicyclooctane and transesterification of monoacetate. Conditions were, therefore, employed which should deter the intermolecular reaction (transesterification) and encourage intramolecular reaction (cyclisation). Indeed the reactions where solvent was not used greatly favoured intermolecular reactions. Thus, very slow addition of triol monoacetate to a large amount of refluxing xylene was tried. However, transesterification occurred even under these conditions and a mixture of triol and its acetates were obtained. No further time was available to investigate the preparation of trioxabicyclooctanes efficiently from carboxylic acids.

3.10 ATTEMPTED FORMATION OF ORGANOMETALLIC COMPOUNDS FROM 4-(3'-HALOPROPYL)-1-METHYL-2,6,7-TRIOXABICYCLO[2,2,2]OCTANES

(a) Attempted Formation of a Grignard Reagent

Attempts to form a Grignard reagent from orthoester (3b) using normal conditions failed. All combinations of ether and THF as solvent and heat, iodine, methyl iodide and dibromoethane as initiators were used with no sign of reaction of the orthoester with magnesium in any case. Because the solvent was dried with lithium aluminium hydride and the i.r. spectrum of the starting material indicated no contamination with normal ester or water, it must be assumed that the orthoester was simply too unreactive to form a Grignard reagent. The ease of formation of Grignard reagents from alkyl or aryl halides, RX, depends on X: (usually X = Cl < Br < I). However, 4-(3'-iodopropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (3i) also failed to produce a Grignard reagent under a variety of conditions similar to those used with the chloro-analogue (3b).

There seemed to be little effect from changing the solvent (orthoesters 3b and 3i were as unreactive in ether as the more strongly coordinating and higher boiling THF) or initiator, so a more active form of magnesium was used. Rieke and Bales[174] have prepared magnesium in a highly active form by reduction of magnesium chloride in THF with potassium. Refluxing of these materials gave a very fine black powder of magnesium, conveniently suspended in THF so that organic halide could be added directly. The magnesium prepared in this way was extremely reactive towards alkyl and aryl halides. For example, bromobenzene reacted with 'Rieke magnesium' to give phenylmagnesium bromide in a few minutes at -78°. The origin of this high reactivity is unknown. It is thought that one or more of the following factors may be important: particle size, surface area, high energy crystal packing, lack of oxide coating, the

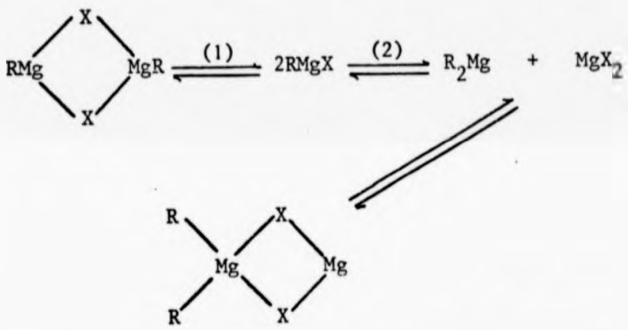
presence of alkali metal salt. Surprisingly, orthoester (3b) failed to react with 'Rieke magnesium' and pure starting material was recovered from the reaction.

(b) Mechanism of Grignard Reactions

To understand or offer an explanation as to why the Grignard reagent of orthoesters (3b) and (3i) failed to form, the theory of the mechanism of formation of Grignard reagents needs to be discussed.

There has been prolonged controversy concerning the nature of Grignard reagents in solutions. Discordant results have often been obtained because of failure to eliminate impurities such as traces of water or oxygen, which can aid or inhibit the attainment of equilibrium and the occurrence of exchange reactions. Recent work[175,176] has given a reasonable understanding of the mechanism by which Grignard reagents form under strict conditions, but those prepared without special precautions may behave differently. X-ray diffraction studies on crystalline phenyl- and ethyl magnesium bromide show that the magnesium atom is essentially tetrahedrally surrounded by an alkyl (aryl) group, bromide, and two oxygen atoms of ether. For less sterically demanding ethers, such as THF, higher co-ordination numbers may occur as in methyl magnesium bromide, which contains three THF molecules. Thus, it is now clear that in crystals the basic Grignard structure is RMgX·n (solvent). However, the nature of this complex in solution is complicated, depending on the nature of the alkyl and halide groups, and on the solvent, concentration, and temperature. Generally, the equilibria are of the type shown in Scheme 3.6 where solvation is not shown. Association is predominantly by halide rather than carbon bridges, although bridging methyls may occur. In dilute solutions

SCHEME 3.6



A possible equilibrium mixture of magnesium species formed during a Grignard reaction

and in more strongly donor solvents, the monomeric species (equilibrium (2)) normally predominate, but in diethyl ether at concentrations of greater than 0.1 M, association occurs and linear or cyclic polymers may be present. Co-ordination of the solvent to the magnesium also accounts for the high solubility of Grignard reagents in ethers. The mechanism by which the magnesium metal atom interacts with the halogen atom of a Grignard precursor is virtually unknown, although it is thought to entail a single electron transfer and hence involve radicals.

(c) Reactions of Orthoesters with Grignard Reagents

Further insight into the inactivity of orthoesters (3b) and (3i) to magnesium may be obtained by looking at some of the known reactions of orthoesters with Grignard reagents.

Many orthoformates have been reacted with Grignard reagents to yield acetals and aldehydes (Scheme 3.7). The synthesis of aldehydes from Grignard reagents and orthoformates is known as the Boudroux-Chichibabin reaction after the chemists who independently discovered the reaction in 1904.

Higher trialkyl orthocarboxylates have also been reacted with Grignard reagents to form ketals from which ketones are obtained by acidic hydrolysis (Scheme 3.8). The first reaction of this kind was reported by Blaise and Marie[177] who converted ethyl triethoxyacetate to 4-ethyl-4-hydroxy-3-oxohexane by reacting it with ethyl magnesium iodide and hydrolysing the initial product. Triethyl orthopropionate and triethyl orthovalerate have also been converted into ketones[178]. Yields have ranged from 6% to 50%.

The mechanism of the reaction between orthoesters and Grignard reagents is not well understood. The first step of the reaction is thought to be a complex formation between the orthoester oxygen atoms and electrophilic magnesium atoms of the various species

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SCHEME 3.7

$$RC(OEt)_3 + \overset{'}{R}MgX \longrightarrow \overset{'}{R}CR(OEt)_2 + MgXOEt$$

$$\overset{'}{R}CR(OEt)_2 + H_2O \xrightarrow{H^+} \overset{'}{R}CRO + 2EtOH$$

R = H, alkyl

Reaction of orthoesters with a Grignard reagent

SCHEME 3.8

R• = radical species

4-Alkyl-1-methyl-2.6.7-trioxabicyclo[2,2,2]octane

Acting as a radical trap.

present in the Grignard solution (R_2Mg, RMgX and MgX_2). Products are then formed either by reaction of the dialkoxy carbonium ion produced by dissociation of this complex with an organomagnesium species or by concerted displacement of the complexed alkoxy group by a carbanion group from the Grignard reagent.

(d) Theories about the lack of Reactivity of 4-(3'-Chloro (or Iodo)Propyl)-1-Methyl 2,6,7-Trioxabicyclo[2,2,2]Octane Towards Metals

There are three possible explanations for the failure of bicyclic orthoesters (3b) and (3i) to form a Grignard derivative:

(i) The 2,6,7-trioxabicyclo[2,2,2]octane group may effect in some way the nearby halogen atom of the propyl substituent.

(ii) The 2,6,7-trioxabicyclo[2,2,2]octane group may act as a radical trap because it contains a quaternary carbon atom, one of the bonds of which, if broken homolytically, would yield a very stable tertiary radical (see Scheme 3.9).

(iii) The 2,6,7-trioxabicyclo[2,2,2]octane group is a rigid cage structure containing three oxygen atoms. It may be that the group can sequester a magnesium species binding it with electron pairs of the fixed oxygen atoms.

(e) Assessment of the Validity of Theories for the Lack of Reactivity of 2,6,7-Trioxabicyclo[2,2,2]Octanes Towards Magnesium

If a 2,6,7-trioxabicyclo[2,2,2]octane is synthesised bearing a long chain substituent with a terminal halide group, then any effect the orthoester function has on the halogen will be minimised by their remoteness from each other. Thus, bromoundecanoic acid, a readily available and cheap material, was converted to its nitrile analogue by a standard method. The acid was refluxed with thionyl

chloride and the resulting acid chloride was treated with ammonia solution to give the amide. Dehydration of the amide by refluxing in thionyl chloride afforded the desired 11-bromoundecanonitrile. A 2,6,7-trioxabicyclo[2,2,2]octane derivative was prepared from this nitrile in the usual way. The nitrile was converted to 11-bromo-1,1,1-triethoxyundecane *via* the iminoester hydrochloride and this was treated with 1,1,1-*tris*(hydroxymethyl)ethane.

The resulting 4-(10'-bromodecyl)-1-methyl-2,6,7-trioxabicyclo [2,2,2]octane, though obtained in pure form, also failed to react with magnesium under normal conditions for Grignard formation in THF, using a variety of initiators. A check on the conditions used for these experiments was made by performing a second preparation using bromooctane as a model for 4-(10'-bromodecyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane. The same conditions as those used in the earlier experiments readily gave a Grignard reagent (reaction warmed and initiated with a small iodine crystal). Addition of acetone to this Grignard reagent caused an exothermic reaction and work-up afforded pure 2-methyldecan-2-ol - easily identified by its distinctive ^{1}H n.m.r. spectrum.

These results indicate that the lack of reactivity of orthoesters (3b) and (3i) is not due to an effect of the orthoester function upon the halogen atom (i.e. explanation i above is invalidated).

Magnesium(II) shows an appreciable tendency to form chelated complexes, and in solution with few exceptions these are oxygen ligands. Many polyether complexes of Mg(II) are known and have been used as models for antibiotics that transport alkaline earth ions across membranes. The fact that magnesium readily chelates with oxygen ligands is illustrated by the solvation of Grignard reagents by ethereal solvents. Also, the known reaction of Grignard reagents with orthoesters shows that magnesium may complex with oxygen atom(s) of

the orthoester. The chelation chemistry of magnesium suggests that the 2,6,7-trioxabicyclo[2,2,2]octane group complexes with magnesium species and somehow prevents Grignard formation. In attempts to form Grignard reagents from orthoesters (3b) and (3i), the same weight of magnesium used at the start of the reaction was recovered from the reaction mixture. If Grignard formation were inhibited by complexation of magnesium atoms to the 2,6,7-trioxabicyclo[2,2,2]octane group, then a loss in weight of magnesium might be expected.

Because no loss of magnesium took place during attempts to prepare Grignard reagents from orthoesters (3b) and (3i), it may be assumed that the reactions are being inhibited at a very early stage, perhaps during the interaction of a magnesium atom with the halide group of the orthoester. A single electron transfer process may be blocked by the 2,6,7-trioxabicyclo[2,2,2]octane group acting as a radical trap. Without the use of sophisticated techniques, this postulate cannot be proved. However, an indication as to whether this proposal is correct may be gained by adding orthoester (3b) or (3i) to a Grignard reaction that is known to occur in the absence of orthoester. This experiment was not done due to lack of time.

(f) Attempted Formation of a Copper Lithium Derivative from 4-(3'-Chloropropyl)-1-Methyl-2,6,7-Trioxabicyclo[2,2,2]octane

Alkyllithium derivatives are normally prepared by a similar technique to that employed in preparing Grignard reagents; yields are usually excellent and the reaction with lithium starts more readily and proceeds at a greater rate than with magnesium. Alkyl and aryllithiums react readily with oxiranes without the need for heating.

Numerous reactions, which have failed with Grignard reagents, have been carried out with alkyllithium derivatives.

Alkyllithium can be reacted with copper(I) iodide to give

dialkylcopper lithium derivatives which have a number of advantages over both Grignard reagents and alkyllithiums (i.e. less reactive and produce higher yields with less coupled product).

When orthoester (3b) was stirred under nitrogen with lithium shavings in ether, no reaction took place and even after 4 hours of boiling at reflux, a lithium derivative was not obtained. To check the conditions used, a similar preparation was carried out using chlorobutane as a model compound. This reaction was carried out by adding an ethereal solution of chlorobutane to freshly prepared lithium shavings in ether, whilst gentle reflux was maintained. After addition of copper(I) iodide, reaction with methyloxirane gave 83% heptan-2-ol.

It therefore appears that orthoester (3b) inhibits the formation of an alkyllithium derivative.

Metal-halogen exchange between a simple alkyllithium compound and orthoester (3b) was not attempted in view of the fact that such exchange reactions involving simple alkyl halides, are not usually useful. The low electronegativity of the alkyl groups lead to an unfavourable equilibrium and substantial competition exists from coupling and elimination reactions[179]. The reaction between ethyllithium and methyl iodide in benzene is an exception as the equilibrium is displayed by precipitation of the insoluble methyllithium[180].

3.11 CONCLUSION

There are two problems which severely restrict the use in organic synthesis, of 2,6,7-trioxabicyclo[2,2,2]octanes as masked forms of carboxylic acids. These are:

(1) The route to 2,6,7-trioxabicyclo[2,2,2]octanes from nitriles is long, tedious and, proceeds in fairly low overall yield. Such a synthesis is only suitable for specialised cases, such as the preparation of cobaloximes with ester or acid groups. For the masking group to be

of more general use, a means of forming it directly from an acid is needed.

(2) The surprising failure of orthoesters (3b) and (3i) to form either a Grignard or alkyllithium derivative.

Although much work had been done on the assessment of 2,6,7-trioxabicyclo[2,2,2]octanes, it was eventually felt that work on it should be suspended and an alternative means of carboxylic acid masking should be used for the synthesis of lipoic acid. Of the ones discussed in Chapter 2, the most attractive was the use of a 2-substituted-2-methyl-1,3-dioxolane.

B. THE USE OF 5-CHLOROPENTAN-2-ONE IN THE SYNTHESIS OF LIPOIC ACID

Numerous methyl ketones have been protected as 1,3-dioxolanes[181,148-150] and 2-haloalkyl-2-methyl-1,3-dioxolanes have been used successfully in Grignard reactions. 2-(3'-Chloropropyl)-2-methyl-1,3-dioxolane (3j) was prepared from ethyl acetoacetate essentially as described[146,147,182]. In order to check the viability of this compound for the synthesis of lipoic acid, a number of model experiments were conducted. First of all, its Grignard reagent was made in THF using normal conditions and dibromoethane as initiator. The formation of Grignard reagent occurred fairly readily with the disappearance of most of the magnesium. To test that the Grignard reagents had indeed been formed, the reaction mixture was worked up with aqueous ammonium chloride. The expected 2-methyl-2-propyl-1,3-dioxolane was obtained, (identified by ^{1}H n.m.r. spectroscopy). The Grignard reagent from dioxolane (3j) reacted readily with methyloxirane in the presence of catalytic dilithium tetrachlorocuprate. The resulting 2-(5'-hydroxyhexyl)-2-methyl-1,3-dioxolane was characterised

by its spectral data and was obtained in a fairly pure state in 72% yield. The organomagnesium derivative of dioxolane (3j) also reacted with the tosylate of propan-1-ol in the presence of dilithium tetrachlorocuprate, giving 2-hexyl-2-methyl-1,3-dioxolane in 70% yield, identified by ^{1}H n.m.r. spectroscopy and confirmed by m.p. of the D.N.P. derivative of the ketone from hydrolysis of the dioxolane function.

EXPERIMENTAL

SYNTHESIS OF NITRILES FOR PREPARATION OF 4-ALKYL-1-METHYL-2,6,7-TRIOXABICYCLO[2,2,2]OCTANES

11-Bromoundecanoamide was prepared as a white solid from 11-bromoundecanoic acid by standard means[183] to give a white powder which was washed with water then dried in a vacuum deciccator (10 mmHg) over phosphorus pentoxide to give 11-bromoundecanoamide (in 83% yield). m.p.87.5.

N.m.r. ($CDCl_3$, TMS): 1.29 (br.s, 10 H, $-(CH_2)_5-$), 1.63-1.90 (m, 6 H, Br $CH_2(CH_2)_3CH_2-NH_2$) 8.21 (t, 2 H, $CH_2CH_2CONH_2$, 3.40 (t, 2 H, $-CH_2Br$), 4.79 (br.s, 1 H, -NH), 5.45 (br.s, 1 H, -NH).

11-Bromoundecanonitrile was prepared by dehydration of 11-bromoundecanoamide with thionyl chloride in the usual way[184]. A colourless oil of 11-bromoundecanonitrile was obtained in 84.3% yield and purified by distillation b.p. 97°, 0.001 mmHg pure by i.r. (film) 2920 (s), 2850 (s), 2240 (m), 1465 (s), 1425 (m), 1370 (w), 1350 (w), 1300 (w), 1275 (w), 1255 (m), 1210 (w), 720 (m).

N.m.r. ($CDCl_3$, TMS): 1.30 (br.s, 8 H, 4X-CH_2), 1.44 (m, 4 H, 2 x-CH_2), 1.67 (p, 2 H, $CH_2CH_2CH_2CN$), 1.85 (p, 2 H, $-CH_2CH_2Br$), 2.34 (t, 2 H, $-CH_2CN$), 3.41 (t, 2 H, $-CH_2Br$).

4-Chlorobutyronitrile was prepared by the standard method[185] from 1-bromo-3-chloropropane in 65% yield, as a colourless oil, b.p. 92-94°, 25 mmHg.

N.m.r. (CCl_4, TMS) 2.09 (t, 2 H, $-CH_2CH_2CH_2^-$), 2.53 (t, 2 H, $-CH_2CN$), 3.64 (t, 2 H, $-CH_2Cl$).

SYNTHESIS OF 4-ALKYL-1-METHYL-2,6,7-TRIOXABICYCLO [2,2,2]OCTANE DERIVATIVES

A. GENERAL PROCEDURE FOR PREPARATION OF IMINOESTER HYDROCHLORIDES

Dry ethanol (400 ml) was placed in a r.b. flask, protected from moisture and cooled to 0° (ice-slush bath). To the cold ethanol was added, dropwise, acetyl chloride (1.25 mol) over 30 min, followed by the nitrile (1 mol). The mixture was left for the appropriate amount of time (Table 3.2) at 0° whilst white crystals formed. After this period of time, dry ether (400 ml) was added to the mixture and shaken (to assist in filtration of crystals). The mixture was then cooled to -78° (dry ice-acetone bath) and the crystals quickly filtered off and washed with dry ether. The salt was dried in a vacuum desiccator (10 mmHg) over sodium hydroxide pellets for 3 h then pumped overnight at 0.001 mmHg. A white crystalline product of ethyl iminoalkylate hydrochloride was obtained (Table 3.2).

Ethyl iminoacetate hydrochloride

N.m.r. (D_2O, DSS): 1.19 (t, 3 H, $-OCH_2\underline{CH_3}$), 2.25 (s, 3 H, $\underline{CH_3}C=O$), 4.39 (q, 2 H, $-OCH_2CH_3$).

Ethyl 4-chloroiminobutanoate hydrochloride

N.m.r. (CD_3OD, TMS): 1.48 (t, 3 H, $OCH_2\underline{CH_3}$), 2.19 (p, 2 H, $-CH_2\underline{CH_2}CH_2-$), 2.87 (t, 2 H, $CH_2\underline{CH_2}C=N$), 3.68 (t, 2 H, $-\underline{CH_2}Cl$), 4.47 (q, 2 H, $-OCH_2CH_3$), 5.16 (br.s, 2 H, $=\overset{+}{N}\underline{H_2}$). M.p. 103-104° (dec.).

Ethyl 11-bromoiminoundecanoate hydrochloride

N.m.r. (CD_3COD, TMS): 1.34 (brs, 12 H, $6\times\underline{CH_2}$), 1.49 (t, 3 H, (CH_3), 1.72 (p, 2 H, $\underline{CH_2}CH_2C=N-$), 1.85 (p, 2 H, $-\underline{CH_2}CH_2Br$), 2.68 (t, 2 H,

$\underline{CH_2}C{=}N-$), 3.44 (t, 2 H, $\underline{CH_2}Br$), 4.47 (q, $-O\underline{CH_2}CH_3$), 4.88 (s, 2 H, $-\underline{NH_2}$).

B. GENERAL PROCEDURE FOR THE ALCOHOLYSES OF IMINOESTER HYDROCHLORIDES

Ethyl iminoalkylate hydrochloride (0.1 mol) was dissolved in 73 ml (1.2 mol) dry ethanol in a r.b. flask which was then stoppered. The solution was left for 3 days at r.t., whilst ammonium chloride precipitated as a white solid. Dry ether (73 ml) was added to the reaction mixture which was then cooled to 0° (ice-slush bath). The solid material was filtered off and the filtrate washed with sodium carbonate solution (10%, 73 ml) and then with a saturated solution of sodium carbonate (73 ml). The organic fraction was then dried (potassium carbonate) and the solvent removed to give a colourless oil of the 1,1,1-triethoxyalkane. If the product contained normal ester as an impurity, it was removed by the following procedure:

To the crude 1,1,1-triethoxyalkane in dichloromethane (50 ml) was added benzyltri-n-butylammonium bromide (1.5 g), and aqueous sodium hydroxide (2 M, 50 ml). The mixture was stirred vigorously for 3 h and then the organic layer was separated, washed with saturated solution of sodium carbonate (50 ml), dried (anhydrous potassium carbonate) and evaporated to yield a colourless oil of pure orthoester (Table 3.3).

1,1,1-Triethoxyethane

N.m.r. (CCl_4, TMS): 1.12 (t, 9 H, $3\times O\underline{CH_2}\underline{CH_3}$), 1.35 (s, 3 H, $\underline{CH_3}-C-$), 3.42 (q, 6 H, $3\times -O\underline{CH_2}CH_3$). b.p. 144.

4-Chloro-1,1,1-triethoxybutane

I.r. (film): 2970 (s), 2920 (m), 2880 (m), 1455 (m), 1390 (w), 1365 (w), 1300 (w), 1280 (w), 1215 (s), 1180 (w), 1050 (s), 995 (w), 955 (w) (no ester peak).

TABLE 3.2

Preparation of R-C(=$\overset{+}{N}H_2Cl^-$)-OC_2H_5

R	Reaction Time	Yield	Starting Material
CH_3	1 day	69.6%	Acetonitrile
$ClCH_2(CH_2)_2$	2 days	87.4%	Chlorobutyronitrile
$BrCH_2(CH_2)_9$	3 days	50.5%	11-Bromoundecanonitrile

TABLE 3.3

Preparation of $RC(OEt)_3$

R	Reaction Time/Days	Yield %	% Normal ester	b.p.
CH_3	3	49	0	$144\text{-}146^{\circ}_{760}$
$ClCH_2(CH_2)_2$	3	73	5	$41\text{-}43^{\circ}_{0.001}$
$BrCH_2(CH_2)_9$	4	89	0	-

N.m.r. (CCl_4, TMS): 1.15 (t, 9 H, 3x-$OCH_2\underline{CH_3}$), 1.81 (br.s, 4 H, $ClCH_2\underline{CH_2}\underline{CH_2}$-), 3.49 (q, 8 H, -$\underline{CH_2}Cl$ and 3x-$OCH_2\underline{CH_3}$).

11-Bromo-1,1,1-triethoxyundecane

N.m.r. (CCl_4, TMS): 1.13 (t, 9 H, 3x-$OCH_2\underline{CH_3}$), 1.29-1.70 (brm, 16 H, 8x$\underline{CH_2}$), 1.84 (t, 2 H, -$\underline{CH_2}CH_2Br$), 3.33 (t, 2 H, -CH_2Br), 3.43 (q, 6 H, 3x-$OCH_2\underline{CH_3}$)

C. GENERAL PROCEDURE FOR THE PREPARATION OF 4-ALKYL-1-METHYL-2,6,7-TRIOXABICYCLO[2,2,2]OCTANES

1,1,1-Triethoxyalkane (0.1 mol) and 1,1,1-*tris*(hydroxymethyl)ethane (0.1 mol) were refluxed overnight in benzene (60 ml). After the reaction mixture had been allowed to cool, the solvent was removed to give a residue of crude product:

1,4-Dimethyl-2,6,7-trioxabicyclo[2,2,2]octane

Crude bicyclic orthoester was obtained as a white solid which was sublimed in a cold finger apparatus at 70° and 0.05 mmHg. Colourless cubic crystals of pure product were obtained in 75.8% yield m.p. 105°.
Pure by: t.l.c. [chloroform, iodine, R_F 0.52].
I.r. (mull): 2925 (s), 2880 (s), 1460 (m), 1400 (s), 1355 (s), 1270 (s), 1205 (m), 1185 (m), 1120 (s), 1050 (s), 935 (m), 860 (s).
N.m.r. (CCl_4, TMS): 0.78 (s, 3 H, $\underline{CH_3}CCH_2O$), 1.45 (s, 3 H, $\underline{CH_3}$ C-O-), 3.89 (s, 6 H, 3x$\underline{CH_2}$).

4-(3'-Chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane

The crude orthoester was obtained as a viscous colourless oil, b.p. 94-96° at 0.001 mmHg. The oil was left to stand at 0° for a few hours and white crystals formed. The solid material was recrystallised from pentane at -15° (ice-salt bath) to give a white solid of product in 66% yield m.p. 25°.

Pure by: t.l.c. [diethyl ether, iodine, R_F 0.59].

I.r. (melt): 2950 (m), 2900 (m), 2870 (s), 1490 (w), 1470 (w), 1460 (m), 1445 (m), 1400 (s), 1380 (w), 1350 (m), 1325 (m), 1260 (m), 1200 (m), 1190 (m), 1170 (m), 1055 (s), 1010 (w), 990 (s), 960 (m), 950 (m), 900 (m), 780 (m), 760 (m).

N.m.r. (CCl_4, TMS): 0.80 (s, 3 H, $\underline{CH_3}$) 1.71 (t, 2 H, α $\underline{CH_2}$), 1.88 (m, 2 H, β CH_2), 3.51 (t, 2 H, $\underline{CH_2}Cl$), 3.81 (s, 6 H, 3x-OCH_2-).

4-(10'Bromodecyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane

The crude product was obtained as a white solid which was recrystallised from pentane to give white crystals of pure orthoester, in a 67% yield m.p. 58-50.

Pure by: i.r. (melt) 2960 (s), 2910 (s), 2860 (s), 2725 (w), 2690 (w), 1490 (m), 1480 (s), 1460 (s), 1400 (m), 1375 (m), 1365 (m), 1350 (m), 1315 (w), 1280 (m), 1265 (w), 1240 (m), 1225 (w), 1215 (m), 1190 (m), 1155 (m), 1105 (w), 1060 (s), 1040 (s), 1020 (s), 990 (s), 965 (s), 935 (m), 915 (m), 890 (m), 870 (m), 820 (w), 765 (w), 720 (m), 710 (m).

N.m.r. (CCl_4, TMS): 0.76 (s, 3 H, -$\underline{CH_3}$), 1.26 (br.s, 12 H, 6x$\underline{CH_2}$), 1.45 (m, 4 H, 2xCH_2), 1.82 (p, 2 H, $BrCH_2\underline{CH_2}$-), 3.3 (t, 2 H, $Br\underline{CH_2}$-), 3.75 (s, 6 H, 3x-OCH_2-).

1-Methyl-2,8, 9-trioxa-adamantane

To a solution of cyclohexane-1,3,5-triol* (0.46 g, 3.5 mmol), in dry ethanol (3 ml) was added 1,1,1-triethoxyethane (0.41 g, 3.6 mmol) and 1 drop of boron trifluoride diethyl etherate. The colourless solution was stirred overnight turning yellow. Evaporation of the solvent afforded yellow crystals which were taken up in ethanol free chloroform and the insoluble yellow material filtered off and discarded. The chloroform solution was filtered through basic alumina and then

*A kind gift from P. Lampe, Department of Chemistry and Molecular Sciences, University of Warwick

evaporated to yield 0.25 g of white crystals.

The product was sublimed in a cold finger apparatus at 85° (oil bath), to give white crystals (0.207 g, 32%).

N.m.r. (CCl_4, TMS): 1.24 (s, 3 H, CH_3), 1.58 (d, 3 H, J = 8 Hz, 3 x eq H' of ring CH_2), 2.70 (d, 3 H, J = 8 Hz and 2 H, 3 x ax H' of ring CH_2), 4.24 (brs, 3 H, 3 x methine ^1H of ring).

KINETICS EXPERIMENTS ON THE HYDROLYSIS OF VARIOUS ORTHOESTERS

MATERIALS

p-Dioxane (spectrophotometric grade) was purified by the method of Perrin, Amego and Perrin[186] and stored in the dark under nitrogen. All other reagents were of Analytical grade.

Stock Buffer Solution - Acetic acid - sodium acetate buffer solution of constant ionic strength was prepared from standardised 0.5 M aqueous solutions of acetic acid (16.70 ml), sodium hydroxide (8.35 ml) and sodium chloride (15.65 ml) which were combined and made up to 50 ml. The resulting buffer solution had a pH of 4.66.

Reaction Solutions - were prepared by pipetting 5 ml of the stock buffer solution and 14.628 ml of dioxane into a 25 ml volumetric flask and filling the flask to the calibration mark with distilled water. The resulting solution consisted of 60.4% dioxane, $R = [HA]/[A^-] = 1$ $[HA] = [A^-] = 1.67 \times 10^{-2}$ M and the ionic strength was maintained at 4.8×10^{-2} M with the sodium chloride.

Kinetic Measurements - The reactions were monitored spectroscopically by following the appearance of the u.v. band of the diol-ester product at 225 nm. An SP1800 recording spectrophotometer equipped with a thermostatted cell holder was used. An experiment was started by allowing 3 ml of the reaction solution in a silica absorption cell to reach thermal equilibrium with the spectrophotometer, then adding 10 µl of orthoester to the cell, quickly inverting the cell and replacing in the cell holder. Optical absorbance was then recorded as a function of time.

Calculations - First order rate constants were computed from plots of

(log $A_\infty - A_t$) *vs.* t and the half-life was obtained from the formula $t_{\frac{1}{2}} = 0.693/k$. Results are given in Table 3.1

2,2-*bis*(Hydroxymethyl)propyl 4 -chlorobutanoate

4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (0.5 g, 2.5 mmol) was weighed into a conical flask and an aqueous solution of 0.05 M hydrochloric acid (5 ml) was added. Vigorous stirring by means of a magnetic follower, produced a cloudy emulsion which after 30 secs. turned instantly clear. The aqueous solution was then extracted with ether (3 x 5 ml) and the organic extracts were combined, dried (magnesium sulphate) and evaporated to give a colourless oil (0.49 g, 98%).

T.l.c. [ether, iodine, R_F 0.258]

I.r. (film): 3400 (v.br.s), 2960 (m), 2930 (sh), 2870 (m), 1730 (v.s), 1465 (m), 1440 (m), 1415 (m), 1385 (m), 1315 (sh), 1295 (m), 1240 (m), 1210 (m), 1145 (m), 1040 (s), 780 (w).

N.m.r. ($CDCl_3$, TMS): 0.84 (s, 3H, $-CH_3$), 2.10 (p, 2 H, $CH_2CH_2CH_2$), 2.55 (t, 2 H, $ClCH_2CH_2CH_2-$), 3.23 (br.s, 2 H, 2XOH), 3.54 (d, 4 H, 2X CH_2OH), 3.61, (t, 2 H, $ClCH_2$), 4.15 (s, 2 H, $O-CH_2-C$).

Stability of 2,2-*bis*(hydroxymethyl)propyl 4 -chlorobutanoate

The compound underwent thermal decomposition during an attempted distillation at 190°, 0.025 mmHg to produce a white solid and colourless oil.

The oil was dissolved in hexane, ether (1:1, 5 ml) and the white solid filtered off and retained. The filtrate was evaporated to give an oil consisting of two products; t.l.c. [ether, iodine, R_F 0.25 = the mono ester, 2,2-*bis*(hydroxymethyl)propyl-4 -chlorobutanoate and R_F 0.44 = the diester (2,2-*bis*(4 -chlorobutoxymethyl)propan-1-ol).

The ^1H n.m.r. spectrum of the oil showed identical peaks to those of the starting material, 2,2-*bis*(hydroxymethyl)propyl 4 -chlorobutanoate. The remaining peaks confirmed the presence of the diester, n.m.r. ($CDCl_3$, TMS): 0.98 (s, 3 H, $C\underline{H}_3$), 2.11 (m, 4 H, 2x-$CH_2C\underline{H}_2CH_2$), 2.56 (m, 4 H, -$CH_2C\underline{H}_2O_2$-), 3.05 (br.s, 1 H, $O\underline{H}$), 3.62 (s, 2 H, $C\underline{H}_2OH$), 3.65 (t, 2 H, $ClC\underline{H}_2$-), 4.07 (s, 4 H, 2 xO-$C\underline{H}_2C$-).

The white solid was washed with hexane then dried *in vacuo*. The n.m.r. spectrum showed it to be 1,1,1-*tris*(hydroxymethyl)ethane. N.m.r. (CD_3OD, TMS): 0.80 (s, 3 H, $C\underline{H}_3$), 3.47 (s, 6 H, 3 x $C\underline{H}_2$), 4.85 (s, 3 H, 3 x $O\underline{H}$).

The ratio of the mono- and diester products was found, by integration of the n.m.r. spectrum of the oil, to be 1:2 respectively.

Pure 2,2-*bis*(hydroxymethyl)propyl 4 -chlorobutanoate was left for 3 days at room temperature after which time it had formed a white sludge which was separated into a white solid of 1,1,1-*tris*(hydroxymethylethane) and an oil of mono- and diester.

Even when stored at 0°C the product decomposed over a few weeks.

<u>γ-Butyrolactone from 2,2-*bis*(hydroxymethyl)propyl 4 -chlorobutanoate by base hydrolysis</u>

2,2-*bis*(hydroxymethyl)propyl 4 -chlorobutanoate (2.00 g, 1.60 mmol) was dissolved in THF (25 ml). To this solution was added sodium hydroxide (25 ml, 1 M) and the mixture was stirred for 3 h. The reaction mixture was then extracted with dichloromethane (3 x 25 ml) and the extracts dried (anhydrous potassium carbonate) and evaporated to yield a colourless oil which was distilled to give a pure colourless oil (1.13 g, 82.0%) b.p. 82-83°, 10 mmHg.

I.r. (film): 2980 (m), 2910 (m), 1770 (v.s), 1460 (m), 1420 (m), 1375 (m), 1315 (w), 1275 (w), 1235 (m), 1160 (v.s), 1035 (s), 990 (s), 930 (w), 870 (w), 800 (w).

N.m.r. ($CDCl_3$, TMS): 2.30 (q, 2 H, $-CH_2\underline{CH_2}CH_2$), 2.50 (t, 2 H, $\underline{CH_2}C{=}O$), 4.34 (t, 2 H, $\underline{CH_2}OCO$).

γ-Butyrolactone from 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane by neutral hydrolysis

4-(3'-Chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (0.5 g, 2.4 mmol) was added to water (10 ml) and the mixture was refluxed overnight. After this time the mixture was extracted with dichloromethane and the extracts dried (anhydrous potassium carbonate) and evaporated to give a 65% yield of product identical with that obtained by base hydrolysis of 2,2-*bis*(hydroxymethyl)propyl-4-chlorobutanoate (i.e. γ-butyrolactone).

Acetic acid from 1-methyl-2,4,10-trioxa-adamantane by neutral hydrolysis

1-Methyl-2,4,10-trioxa-adamantane was treated as above, and acetic acid was isolated in 61% yield (identified by its ^1H n.m.r. spectrum).

γ-Butyrolactone from 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo-[2,2,2]octane by acid hydrolysis.

4-(3'-Chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (1 g, 4.85 mmol) was added to a 1 M solution of hydrochloric acid (10 ml). The mixture was stirred under reflux overnight and then extracted with dichloromethane (3 x 10 ml). The combined extracts were dried (anhydrous potassium carbonate), and evaporated to give a product identical to that obtained by base and neutral hydrolysis of 2,2-*bis*(hydroxymethyl)propyl-4'-chlorobutanoate.

Ethyl 4-chlorobutanoate

To a solution of 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxobicylco[2,2,2]-octane (0.2 g, 0.97 mmol) in 0.1 M ethanolic hydrogen chloride (5 ml, generated by the addition of the correct amount of acetyl chloride to ethanol) was

added 2 drops of water. The reaction mixture was refluxed overnight and then after cooling to r.t. solid sodium carbonate (50 mg) was added and the mixture was stirred for 10 min. Filtration of the solid and evaporation of the filtrate afforded a colourless oil and white solid mixture. The oil was taken up in hexane/ethylacetate (1:1, 5 ml). The solvent was removed to give an oil (0.104 g, 71.4%) b.p. 186-188°, 760 mmHg.

I.r. (film): 2995 (s), 1740 (s), 1400 (m), 1340 (m), 1320 (w), 1260 (m), 1210 (s), 1200 (s), 1130 (w), 1020 (m), 980 (w), 870 (w), 850 (w), 780 (w).

N.m.r. ($CDCl_3$, TMS): 1.25 (t, 3 H, $\underline{CH_3}$), 2.09 (p, 2 H, $ClCH_2\underline{CH_2}$-), 2.49 (t, 2 H, $-\underline{CH_2}CO_2$-), 3.60 (t, 2 H, $-\underline{CH_2}Cl$), 4.14 (q, 2 H, $-O\underline{CH_2}CH_3$).

Methyl 4-chlorobutanoate

The procedure used for the preparation of ethyl 4-chlorobutanoate was carried out using methanol in place of ethanol. 75-80% yields of pure methyl 4-chlorobutanoate were obtained, b.p. 176-177°, 760 mmHg.

I.r. (film): 2995 (m), 1740 (s), 1420 (m), 1360 (m), 1280 (m), 1200 (s), 1160 (s), 1140 (s), 1050 (w), 1000 (w), 875 (w), 785 (w).

N.m.r. ($CDCl_3$, TMS): 2.11 (p, 2 H, $-CH_2\underline{CH_2}CH_2$), 2.52 (t, 2 H, $-\underline{CH_2}COMe$), 3.61 (t, 2 H, $ClCH_2$), 3.72 (s, 2 H, $-COMe$).

4-(3'-Iodopropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane

Sodium iodide (35.76 g, 0.24 mol) was weighed into a 500 ml r.b. flask and solid sodium bicarbonate (10 g) and a magnetic follower was added. The solid material was dissolved in acetone (250 ml) and 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (5 g, 0.024 mol) was added. The mixture was protected from moisture and stirred at 50°C for 40 hr. After this time the contents of the flask were concentrated and water (250 ml) was added to the residue. The flask was shaken

and the contents extracted with carbon tetrachloride (200 ml). The organic phase was separated, dried (anhydrous potassium carbonate), and evaporated to give a pale green oil. Upon addition of a small amount of pentane and trituration, white crystals were formed. The mixture was cooled to -15^{o} (ice-salt bath) and the white crystals filtered off and dried in a vacuum desiccator (10 mmHg) containing paraffin wax shavings. In this way, pure white crystals were obtained, (4.99 g, 69.3%) m.p. $41-42^{o}$.

I.r. (melt): 2960 (s), 2915 (s), 2870 (s), 1490 (w), 1470 (m), 1460 (m), 1445 (m), 1430 (w), 1400 (s), 1380 (m), 1355 (m), 1325 (m), 1305 (m), 1260 (m), 1230 (s), 1190 (m), 1175 (m), 1055 (s), 1015 (w), 990 (s), 970 (w), 940 (m), 935 (m), 890 (m), 780 (s), 740 (s).

N.m.r. (CCl_4, TMS): 0.79 (s, 3 H, $-CH_3$), 1.63 (t, 2 H, $-CH_2C-O$), 1.92 (p, 2 H, $ICH_2CH_2CH_2-$), 3.16 (t, 2 H, ICH_2CH_2-), 3.79 (s, 6 H, $3 \times -OCH_2-$).

1,4-Dimethyl-2,6,7-trioxabicyclo[2,2,2]octane directly from acetic acid I

Acetic acid (0.24 g, 4 mmol), was added to a suspension of 1,1,1-*tris*(hydroxymethyl)ethane (4.8 g, 40 mmol) in dimethoxy ethane (30 ml). A small amount of 3A molecular sieves and anhydrous p-toluenesulphonic acid (50 mg, 5 mol per cent) were added and the mixture was stirred under reflux. The reaction was followed by t.l.c. The lack of the appearance of a spot at R_F 0.52 (chloroform, iodine) and the persistance of a spot at the origin due to the starting materials, indicated that no reaction had occurred.

1,4-Dimethyl-2,6,7-trioxabicyclo[2,2,2]octane directly from acetic acid II

1,1,1-*Tris*(hydroxymethyl)ethane (5 g, 0.042 mol) was dissolved in the minimum amount of DMSO (15 ml) and anhydrous p-toluenesulphonic acid

(0.37 g, 5 mol%) and acetic acid (2.52 g, 0.042 mol) added. After heating at 80° overnight, water (50 ml) was added and the mixture extracted with ether (3 x 50 ml). The combined extracts were washed with water (3 x 150 ml), then they were dried and evaporated to give acetic acid. No reaction had taken place.

1,4-Dimethyl-2,6,7-trioxabicyclo[2,2,2]octane directly from acetic acid III

A mixture of 1,1,1-*tris*(hydroxymethyl)ethane (12 g, 0.1 mol) and acetic acid (6 g, 0.1 mol) was refluxed in benzene (30 ml) with azeotropic removal of water (Dean and Stark apparatus). When half the theoretical amount of water (for trioxabicyclooctane formation) was given off (1.8 ml after 4 h) the benzene was removed to afford a viscous colourless oil which was taken up in ether (50 ml) and washed with water (2 x 50 ml). After drying of the ethereal solution, the solvent was evaporated to give a colourless oil. Examination of the ^1H n.m.r. spectrum of the product showed the presence of mono- and diacetates of 1,1,1-*tris*(hydroxymethyl)ethane in a ratio of 2:1 respectively.

N.m.r. 1,1,1-*tris*(hydroxymethyl)ethane monoacetate ($CDCl_3$, TMS): 0.86 (s, 3 H, $C\underline{H}_3(CCH_2)_3$-, 2.08 (s, 3 H, O$\underline{Ac}$), 3.56 (s, 4 H, 2X -$C\underline{H}_2$OH), 3.72 (s, 2 H, 2x-$\underline{O}$H), 4.13 (s, 2 H, -$C\underline{H}_2$OAc).

N.m.r. 1,1,1-*tris*(hydroxymethyl)ethane diacetate ($CDCl_3$, TMS): 0.97 (s, 3 H, $C\underline{H}_3(CCH_2)_3$-), 2.09 (d, 6 H, 2 x O$\underline{Ac}$), 3.45 (s, 2 H, -$C\underline{H}_2$OH), 3.72 (s, 1 H, -$\underline{O}$H), 4.03 (s, 4 H, 2x-$C\underline{H}_2$OAc).

The mixture of mono- and diacetates (1 g) was placed in a B-10 r.b. flask and p-toluene sulphonic acid (100 mg) was added. The flask was connected to 2 Kugelröhr flasks and the mixture was heated at 120°, 0.1 mmHg for 30 min. during which a white solid sublimed onto the cooled walls of one of the flasks (0.1 g, 10%). The ^1H n.m.r.

spectrum of the product was identical to that for 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane.

5-Hydroxymethyl-2,2-dimethyl-5-methyl-1,3-dioxane

A solution of 1,1,1-*tris*(hydroxymethyl)ethane (25 g, 0.21 mol) and 2.5 ml concentrated sulphuric acid in acetone (125 ml) was left to stand at room temperature. The reaction was followed by t.l.c. [ethylacetate, iodine, R_F starting triol = 0.17 , product R_F = 0.41]. After 4 h the reaction had gone to completion and a saturated soln. of $NaCO_3$ (125 ml) was added to the reaction mixture which was then extracted with ether (3 x 125 ml). The extracts were combined, washed with water (200 ml), dried (anhydrous potassium carbonate) and evaporated. The resulting oil was distilled to give a product pure by t.l.c. [ethylacetate/iodine, R_F = 0.41], (27.45 g, 82.4%) b.p. 50-51°, 0.1 mmHg.

I.r. (film): 3420 (br.s), 2985 (s), 2935 (s), 2860 (s), 1450 (m), 1370 (s), 1350 (sh), 1250 (m), 1200 (s), 1190 (sh), 1150 (s), 1080 (s), 1030 (s), 990 (sh), 925 (sh), 910 (m), 825 (m).

N.m.r. ($CDCl_3$, TMS): 0.84 (s, 3 H, $C\underline{H}_3$-C), 1.43 (d, 6 H, $C\underline{Me}_2$), 2.43 (br.t, 1 H, -O<u>H</u>), 3.68 (3 x s, 6 H, -$C\underline{H}_2$OH and 2 x -$C\underline{H}_2$O-C).

5-acetoxymethyl-2,2-dimethyl-5-methyl-1,3-dioxane

5-Hydroxymethyl-2,2-dimethyl-5-methyl-1,3-dioxane (21 g, 0.35 mol) was dissolved in pyridine (135 ml). The solution was heated at reflux for 1 hr with acetic anhydride (35.4 ml). The reaction mixture was allowed to cool to r.t. and water (325 ml) was added. The mixture was then extracted with ether (3 x 250 ml). The combined organic fractions were dried (anhydrous potassium carbonate) and evaporated to give a residue containing pyridine. The pyridine was distilled out of the crude product as a fraction b.p. 25-90°C, 30 mmHg. The residue was

distilled at 0.1 mmHg to give a colourless oil (23.4 g, 88.3%) b.p. 74-76°.

I.r. (film): 2990 (m), 2970 (m), 2860 (m), 1740 (v.s), 1455 (w), 1375 (s), 1235 (s), 1210 (s), 1150 (m), 1070 (m), 1030 (m), 910 (w), 825 (w).

N.m.r. ($CDCl_3$, TMS): 0.84 (s, 3 H, $\underline{CH_3}C(CH_2-)_3$), 1.43 (d, 6 H, $C(\underline{CH_3})_2$), 2.09 (s, 3 H, $OCO\underline{CH_3}$), 3.65 (d, 4 H, 2 x CH_2 of dioxane ring), 4.16 (s, 2 H, $-\underline{CH_2}OAc$).

1,1,1-*Tris*(hydroxymethyl)ethane monoacetate

5-Acetoxymethyl-2,2-dimethyl-5-methyl-1,3-dioxane (22.8 g, 0.14 mol) was stirred vigorously with a 0.1 M solution of hydrochloric acid (125 ml). The cloudy emulsion which formed, dispersed after 5 min. of stirring to leave a clear solution which was neutralised with a solution of 1 M sodium hydroxide. The aqueous solution was then extracted with ether (3 x 150 ml). The extracts were dried (magnesium sulphate) and the solvent removed to give a colourless oil (17.0 g, 93.4%) pure by:

N.m.r. ($CDCl_3$, TMS): 0.86 (s, 3 H, $\underline{CH_3}-C(CH_2-)_3$), 2.09 (s, 3 H, $\underline{OAc}$), 3.06 (s, 2 H, 2x-$\underline{OH}$), 3.56 (s, 4 H, 2x-CH_2OH), 4.14 (s, 2 H, $-\underline{CH_2}\underline{OAc}$).

NB The product had to be used straight away as the product decomposed to a white sludge. After 3 days at room temperature the white sludge was shaken with hexane/ethylacetate (1:1) and the white insoluble material filtered off and retained. The filtrate was evaporated to give an oil consisting of, mono- and diesters of 1,1,1-*tris*(hydroxymethyl)ethane in a ratio of 2:1 respectively (*cf.* with products obtained by refluxing 1,1,1-*tris*(hydroxymethyl)ethane with acetic acid and attempted cyclisation reactions of 1,1,1-*tris*(hydroxymethyl)ethane monoacetate). The white solid was washed with hexane and dried *in vacuo*. Its ^1H n.m.r. spectrum showed the compound to be 1,1,1-*tris*(hydroxymethyl)ethane.

Cyclisation of 1,1,1-*tris*(hydroxymethyl)ethane monoacetate by heating without a solvent

1,1,1-*Tris*(hydroxymethyl)ethane monoacetate (0.5 g, 3.08 mmol) was placed in a B-10 r.b. 10 ml flask and p-toluenesulphonic acid (87 mg, 15 mol%) was added. The mixture was heated at 100-105° in a Kugelröhr apparatus at a pressure of 0.1 mmHg. A white solid sublimed onto the cooled surface of a Kugelröhr flask (0.047 g, 6%) m.p. 82-83°. The ^{1}H n.m.r. spectrum of the product was identical to that for pure 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane.

Cyclisation of 1,1,1-*tris*(hydroxymethyl)ethane monoacetate by refluxing in solvent

A solution of 1,1,1-*tris*(hydroxymethyl)ethane monoacetate (4.37 g, 0.027 mol) in benzene (10 ml), containing p-toluenesulphonic acid (0.77 g, 15 mol%) was heated at reflux with azeotropic removal of water. After 4 h the reaction mixture was cooled to r.t. and solid sodium carbonate (1.0 g) added. The mixture was stirred for 10 min. then the solid material was filtered off and the solvent evaporated to yield a viscous oil. The ^{1}H n.m.r. spectrum contained peaks of identical chemical shifts to those of the product obtained when 1,1,1-*tris*(hydroxymethyl)ethane was refluxed with acetic acid. Again in this case the ratio of the monoacetate to diacetate product was 2:1 respectively. The crude reaction mixture was placed in a 10 ml, B-10 flask which was then connected to a Kugelröhr flask (0.25 g, 10%) m.p. 82-84°. The ^{1}H n.m.r. spectrum of the product was identical to that obtained for pure 1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane.

ATTEMPTED FORMATION OF GRIGNARD DERIVATIVES OF 4-ALKYL-1-METHYL 2,6,7-TRIOXABICYCLO[2,2,2]OCTANE DERIVATIVES

GENERAL PROCEDURE

A B-10, 3-necked 25 ml, pear-shaped flask was fitted with a self equilibrating dropping funnel, nitrogen inlet and double surface condenser. Into the flask was placed magnesium turnings gram atom) and dry solvent (5 ml). The apparatus was flushed with (1.5x nitrogen whilst the initiator was added and then the reaction flask was gently warmed.

In all cases no reaction was apparent, even with a large amount of initiator and so a solution of 4-alkyl-1-methyl 2,6,7-trioxabicyclo[2,2,2]octane (2 g, x mol) in dry solvent (5 ml) was added and the reaction mixture refluxed overnight. To test if any reaction had taken place 10% (w/v) aqueous ammonium chloride (10 ml) was added to the reaction mixture which was then stirred for 10 min. Extraction of the mixture with dichloromethane (3 x 10 ml) gave, after drying of the extracts (magnesium sulphate), and evaporation of the solvent, a colourless oil which was examined by ^{1}H n.m.r. spectroscopy. The results are given in table 3.4 - in all cases, only starting material was isolated.

2- Methyldecan-2-ol

To a B-10, 25 ml, 3-necked, pear-shaped flask, fitted with dropping funnel, double-surface reflux condenser, nitrogen inlet, calcium chloride drying tubes and magnetic follower, was added dry THF (50 ml).

The apparatus was put under a positive pressure of nitrogen and then magnesium turnings (0.6 g, 0.025 g-atom) were added.

TABLE 3.4

Conditions for Attempted Formation of the Grignard Derivative of 4-Alkyl-1-methyl-2,6,7-trioxabicyclo[2,2,2]octanes

Alkyl Substituent	Initiator	Solvent	Result
$ClCH_2(CH_2)_2$	I_2	Ether	No reaction
	I_2	THF	No reaction
	EtI	Ether	No reaction
	EtI	THF	No reaction
	$BrCH_2CH_2Br$	THF	No reaction
	EtMgBr	THF	No reaction
$ICH_2(CH_2)_2$	I_2	Ether	No reaction
	I_2	THF	No reaction
	EtI	Ether	No reaction
	EtI	THF	No reaction
	$BrCH_2CH_2Br$	THF	No reaction
	EtMgBr	THF	No reaction
$BrCH_2(CH_2)_9$	I_2	THF	No reaction
	EtI	THF	No reaction
	$BrCH_2CH_2Br$	THF	No reaction

A portion of a solution of bromo-octane (40 g, 0.020 mol) in 10 ml dry THF and a small iodine crystal was added to the reaction mixture which was gently warmed until the reaction commenced. Once the reaction started, the remaining bromo-octane solution was added at a rate sufficient to keep the mixture at reflux. After the addition was complete the mixture was refluxed for 40 min. then allowed to cool to r.t. An excess of acetone was added whereupon a vigorous reaction took place. The reaction mixture was then stirred for 1 h with an aqueous solution of ammonium chloride (10% w/v, 25 ml). The mixture was extracted with ether (3 x 25 ml) and the extracts combined, dried (magnesium sulphate) and evaporated to give a colourless oil (3.2 g, 80.4%). b.p. 106^{o}.

N.m.r. (CCl_4, TMS): 1.01 (t, 3 H, $\underline{CH_3}CH_2\_$), 1.20 (d, 6 H, $HOC(\underline{CH_3})_2$), 1.31 (br.s, 14, 7 x CH_2).

4-(3'-Magnesium chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane using active magnesium

A 100 ml 3-necked flask was fitted with a double surface reflux condenser, dropping funnel, nitrogen inlet and magnetic follower. The apparatus was protected from moisture with calcium chloride guard tubes and a positive pressure of nitrogen. Dry THF (50 ml) was added to the flask followed by purified potassium (1.5 g, 0.038 g-atom) and anhydrous magnesium chloride (0.0214 mol). The mixture was stirred and heated to reflux whereupon reduction occurred quickly to give a dark grey powder suspended in the solvent. The mixture was refluxed for 2 h to ensure complete reaction of potassium and was then cooled at r.t. for 30 min. A solution of 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (4.12 g, 0.02 mol), in dry THF (10 ml) was carefully added. Nothing appeared to happen and the mixture was therefore refluxed overnight. Work up of the reaction by addition of 10% (w/v) aqueous ammonium hydroxide and extraction of the mixture with ether (3 x 50 ml) afforded,

after drying (anhydrous magnesium sulphate) and evaporation of the extracts, a product which was examined by ^{1}H n.m.r. spectroscopy. The product was shown to consist of starting material only.

4-(3'-Lithiumpropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane

A 3-necked 250 ml r.b. flask was equipped with dropping funnel, nitrogen inlet, double-surface reflux condenser and magnetic follower. A steady flow of nitrogen was passed through the apparatus which was also protected by calcium chloride guard tubes. Dry ether (40 ml), and lithium shavings (0.5 g, 71.4 mg-atom) were quickly added to the reaction flask. A portion of a solution of 4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane (4.90 g, 23 mmol) was added to the reaction flask which was heated. When no reaction was apparent, the remainder of the chloride solution was added to the mixture which was then refluxed in the hope that some of the lithium shavings would be consumed. However, after overnight reflux there was no apparent change. The organic solvent was decanted into an aqueous solution of 10% (w/v) ammonium chloride (50 ml) which was then extracted with ether (3 x 75 ml). The extracts were combined, dried (anhydrous potassium carbonate) and evaporated to give only starting material.

Heptan-2-ol

A 3-necked 250 ml r.b. flask was equipped with dropping funnel, nitrogen inlet, double surface reflux condenser, magnetic follower and a drying tube. The apparatus was put under a positive pressure of nitrogen and dry lithium shavings (2.0 g, 0.29 g-atom) were added. The shavings were covered with dry ether and a solution of chlorobutane (11.1 g, 0.12 mol) in dry ether was added during 3 h whilst gentle reflux was

maintained by a warm water bath. Reflux was continued for 1 h after addition of the chlorobutane, then the mixture was cooled to -78^{o} (acetone-dry ice bath) and anhydrous copper(I) iodide (11.43 g, 0.06 mol) was added. The mixture was stirred for 1 h at -78^{o} to ensure complete formation of the copper-lithium derivative and then propylene oxide (1.76 g, 0.03 mol) was added. The mixture was stirred for 1 h still at -78^{o} then placed in an ice-bath and stirred overnight whilst the flask warmed to r.t. Ammonium chloride solution (10% w/v), 80 ml) was added to the reaction flask and the mixture stirred for 1 h. After this time, the mixture was extracted with ether (3 x 80 ml) and the extracts were combined, dried (anhydrous magnesium sulphate) and evaporated to give a pale brown oil (2.92 g, 82.9%).

T.l.c. and ^{1}H n.m.r. spectra revealed the product to consist of mainly heptan-2-ol with a small amount of coupled product, octane.
T.l.c. dichloromethane and methanol, 3:1, iodine, R_F 0.755 = heptan-2-ol.
I.r. (film): 3340 (br.m), 2960 (s), 2920 (s), 2853 (sh), 2850 (m), 1465 (m), 1370 (w), 1140 (w), 1110 (w), 1060 (w), 950 (w), 935 (w).
N.m.r. heptan-2-ol ($CDCl_3$, TMS): 0.89 (t, 3 H, $-CH_2\underline{CH_3}$), 1.18 (d, 3 H, $\underline{CH_3}CHOH-$), 1.28-1.43 (br.m, 8 H, 4 x $-\underline{CH_2}$), 1.91 (br.s, 1 H, $HCO\underline{H}$), 3.80 (p, 1 H, $HOC\underline{H}$).
N.m.r. octane ($CDCl_3$, TMS): 0.89 (t, 3 H, $CH_2\underline{CH_3}$), 1.28 (m, 12 H, 6 x $\underline{CH_2}$).
The integrals showed that the heptan-2-ol product contained 12% of coupled material.

5-Chloro-2-oxopentane

This was prepared from ethyl acetoacetate according to the literature procedure[182].

The sodium salt of ethyl acetoacetate was obtained as a white powder in 70.5% yield, m.p. 168-170 (dec). 2-Acetylbutyrolactone was

prepared as a colourless oil which was purified by fractional distillation to give a 67% yield of pure product b.p. 118°, 10 mmHg.
N.m.r. (CCl_4, TMS): 2.24 (m, 1 H, 1 H of ring CH_2 adjacent to the acetyl function), 2.38 (s, 3 H, $-C\underline{H}_3$) 2.71 (m, 1 H, 1 H of ring CH_2 adjacent to the acetyl function), 3.61 (d,t 1 H, ring methine H), 4.28 (m, 2 H, ring methylene). 5-Chloro-2-oxopentane was prepared as a yellow oil which was purified by fractional distillation to give a 77.8% yield of product 58-60°, 10 mmHg.
N.m.r. (CCl_4, TMS): 1.98 (p, 2 H, $ClCH_2C\underline{H}_2CH_2-$), 2.11 (s, 3 H, $-C\underline{H}_3$), 2.57 (t, 2 H, $-CH_2C\underline{H}_2COCH_3$), 3.54 (t, 2 H, $ClC\underline{H}_2-$).

2-(3'-Chloropropyl)-2-methyl-1,3-dioxolane

This was prepared from 5-chloro-2-oxopentane by a standard method[187] to give a yellow oil. Distillation afforded an 80.7% yield of colourless oil b.p. 80°, 10 mmHg. Pure by:
N.m.r. (CCl_4, TMS): 1.23 (s, 3 H, $C\underline{H}_3-$), 1.77 (m, 4 H, $ClCH_2C\underline{H}_2C\underline{H}_2-$), 3.50 (t, 2 H, $ClC\underline{H}_2CH_2-$), 3.86 (s, 4 H, $-OCH_2CH_2O-$).

Formation of a Grignard reagent of 2-(3'-Chloropropyl)-2-methyl-1,3-dioxolane

2-(3'-Chloropropyl)-2-methyl-1,3-dioxolane (1 g, 6.58 mmoles) was dissolved in dry THF (1.5 ml). The reaction was started by warming the reaction vessel in a water bath and adding 1 crystal of iodine. When the reaction had begun, the remaining dioxolane solution was added at a rate sufficient to keep the mixture refluxing. When the addition was complete, the mixture was refluxed for a further 45 min. to ensure complete formation of the Grignard reagent. The Grignard reagent was decomposed by allowing the reaction mixture to cool and adding 10% aqueous ammonium chloride (10 ml). After 15 min. stirring the mixture was extracted with 3 x 10 ml ether. The fractions were

combined, dried (anhydrous magnesium sulphate) and evaporated to give a yellow oil of almost pure 2-methyl-2-propyl-1,3-dioxolane.

N.m.r. (CCL_4, TMS): 1.03 (t, 3 H, $\underline{CH_3}CH_2$-), 1.30 (s, 3 H, CH_3C-O), 1.30-1.67 (m's, 6 H, 3 x CH_2), 3.95 (s, -OCH_2CH_2O-).

Coupling of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane with methyloxirane

The Grignard reagent of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane (1 g, 6.5 mmol) was prepared in dry THF (3 ml) as before. After the Grignard solution had cooled down to 0° (ice-bath), a solution of dilithium tetrachlorocuprate (0.1 M, 0.2 ml) was added. Subsequent addition of methyloxirane (0.4 g, 7 mmol) caused a vigorous, exothermic reaction to take place. After the reaction had subsided, the mixture was stirred at r.t. for 2 h. Aqueous ammonium chloride (10%, 25 ml) was then stirred with the mixture for 15 min. Extraction of the aqueous solution with ether (3 x 25 ml) afforded, after combination of the organic fractions, drying (anhydrous magnesium sulphate) and removal of the solvent, a colourless oil. Distillation in a Kugelröhr apparatus (0.1 mmHg, 150° oven temp.) produced pure 2-(5'-hydroxyhexyl)-2-methyl-1,3-dioxolane (1.1 g, 72%). Pure by:

I.r. (film): 3430 (brs), 2940 (s), 2870 (s), 1460 (m), 1380 (s), 1340 (w), 1300 (w), 1250 (m), 1220 (w), 1110 (sh), 1085 (sh), 1060 (s), 950 (m), 850 (m), 840 (sh).

N.m.r. ($CDCl_3$, TMS): 1.17 (d, 3 H, $\underline{CH_3}CHOH$), 1.32 (s, 3 H, $\underline{CH_3}C$), 1.43 (m, 6 H, $(\underline{CH_2})_3CH_2CHOH$), 1.64 (m, 3 H, OH and -$\underline{CH_2}CHOH$), 3.78 (m, 1 H, -CHOH), 3.94 (s, 4 H, -OCH_2CH_2-).

Tosylate of n-propanol

This was prepared by the standard procedure[188] to give a thick oil which crystallised overnight in the refrigerator. Recrystallisation of the

solid material gave a white powder of pure tosylate (65%).

N.m.r. (CCl_4, TMS): 1.01 (t, 3 H, $-CH_3$), 1.64 (m, 2 H, $CH_3CH_2CH_2$) 2.44 (s, 3 H, CH_3Ph), 3.89 (t, 2 H, $TsOCH_2-$), 7.27 and 7.69 (4H, 'AB' quartet of aromatic ring).

Coupling of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane with propan-1-ol p-toluenesulphonate

The Grignard reagent of 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane (1 g, 6.5 mmol) was prepared as before in THF. After allowing the solution of Grignard to cool to r.t., propan-1-ol p-toluenesulphonate (1.47 g, 6.5 mmol) was added and the mixture cooled to 0°C (ice-bath) whereupon a solution of dilithium tetrachlorocuprate (0.1 M, 0.2 ml) catalyst was added. The mixture was stirred at r.t. overnight then aqueous ammonium chloride (10%, 20 ml) was added and the solution stirred for 15 min. Extraction of the aqueous solution with ether (3 x 25 ml) afforded, after combination of the fractions, drying (anhydrous magnesium sulphate) and evaporation, a yellow oil which was distilled at 5 mmHg in a Kugelröhr apparatus (oven temp. 125°) to give the product (70%, 0.73 g) 0.90 (t, 3 H, CH_3CH_2-), 1.32 (s on top of m, 13 H, CH_3-C and CH_2 x 5), 3.94 (s, 4 H, $-OCH_2CH_2O-$).

The product contained a small amount of impurity and a check was made as to the identity of the product by making the DNP derivative of it[189].

The DNP was prepared by standard means,[189] the conditions of the reaction causing hydrolysis of the ethylene ketal to produce hexyl methyl ketone. A yellow precipitate of DNP was obtained which was recrystallised to give a yellow powder m.p. 57°C (lit. value for hexyl methyl ketone 58°C)[190].

N.m.r. ($CDCl_3$, TMS): 0.90 (t, 3 H, CH_3CH_2-), 1.35 (m, 6 H, 3 x CH_2), 1.64 (m, 2 H, $CH_2CH_2C=N-$), 2.08 (s, 3 H, $CH_3-C=N-$), 2.43 (t, 2 H,

$-CH_2C=N-$), 7.95 (d, 1 H, ArC-H), 8.39 (d, 1 H, ArC-H), 9.10 (s, 1 H, ArCH).

CHAPTER 3 - REFERENCES

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CHAPTER 4

(2R,3R)-Di-n-BUTYL TARTRATE AS A STARTING MATERIAL FOR THE SYNTHESIS OF (R)-LIPOIC ACID

4.1 INTRODUCTION

(S)- 1,2-Dihydroxybut-3-ene has been synthesised by means of a Wittig reaction between triphenylphosphorane and isopropylidene (R)-glyceraldehyde[191]. Hydrolysis of the isopropylidene function of the product, 2,2-dimethyl-4-vinyl-1,3-dioxolane, in refluxing ethanol/3 M hydrochloric acid produced the desired diol. However, drawbacks to this synthesis are that isopropylidene (R)-glyceraldehyde is obtained in low yield from (S)-mannitol[192], the overall yield is only 41% and (R)-mannitol, which would give (R)-lipoic acid, is not commercially available. It was felt that the use of tartrate esters, as suggested in Chapter 2, might be a better method for preparing optically active 1,2-dihydroxybut-3-ene. Both (R,R)- and (S,S)-tartaric acid esters are readily available.

4.2 SYNTHESIS OF RS-1,2-DIHYDROXYBUT-3-ENE

Optically active 1,2-dihydroxybut-3-ene has been made by reaction of (2S,3S)-(2,3-di-O)-isopropylidene threitol with formic acid and pyrolysis of the resulting formates, but the conditions have not been optimised and the optical purity of the product has not been established[193]. In order to do this, preliminary investigations were conducted using the cheap *meso*-erythritol which could be reacted directly with formic acid rather than spending time to synthesise (2S,3S)-(2,3-di-O)-isopropyliden threitol from (2R,3R)-di-n-butyl tartrate.

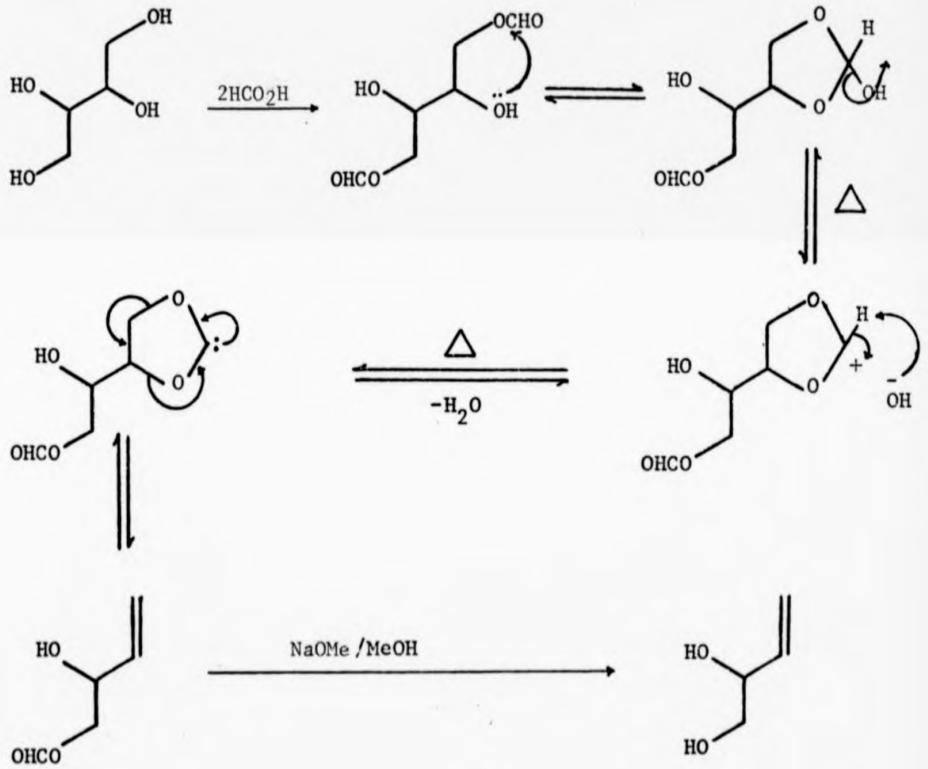
The reaction of *meso*-erythritol with formic acid has always been supposed to produce the 1,4-diformate as the major product, because hydrolysis of the product of its pyrolysis yields 1,2-dihydroxybut-3-ene, Pyrolysis of the 1,4-diformate is envisaged to proceed *via* a cyclic carbene, which decomposes to carbon dioxide and 1,2-dihydroxybut-3-ene 1-O-formate. 1,2-Dihydroxybut-3-ene is obtained by hydrolysis of this formate (Scheme 4.1). When the yellow-brown solid from heating *meso*-erythritol with formic acid was pyrolysed, a distillable colourless oil was obtained in 92% yield (based on erythritol). Methanolysis of this product gave a mixture of two components separable by flash column chromatography.

Two products were obtained in similar amounts and identified by ^{1}H n.m.r. and i.r. spectroscopy as 1,2-dihydroxybut-3-ene and 1,4-dihydroxybut-2-ene There are two methods by which the unwanted 1,4-dihydroxybut-2-ene may have arisen:

(a) Traces of formic acid from the previous reaction may have catalysed the formation of an allylic cation (4a) which could react with water at C-3 to give 1,2-dihydroxybut-3-ene at its terminal carbon atom to give 1,4-dihydroxybut-2-ene (see Scheme 4.2).

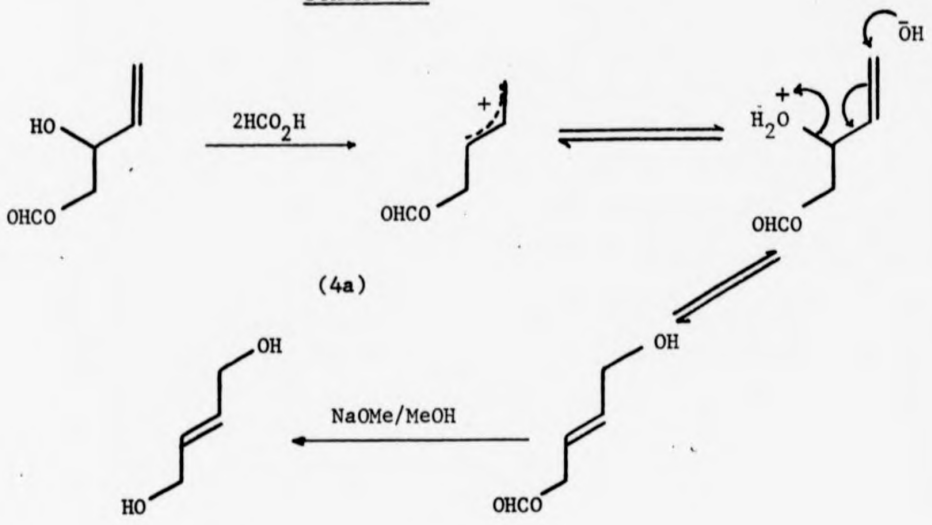
(b) The reaction of *meso*-erythritol with formic acid may have produced not only the 1,4-diformate, but also a substantial amount of 2-(3)-formylated product. Pyrolysis of this compound could cause cyclisation of the formate group in the 2-position with the 3-hydroxy group, leading to 1,4-dihydroxybut-2-ene. When the product from the reaction of *meso*-erythritol and formic acid was pumped in high vacuum overnight to remove all traces of formic acid, pyrolysis followed by hydrolysis gave a product identical to that obtained previously. An acid-catalysed isomerisation was therefore not the cause of the formation of 1,4-dihydroxybut-2-ene.

SCHEME 4.1



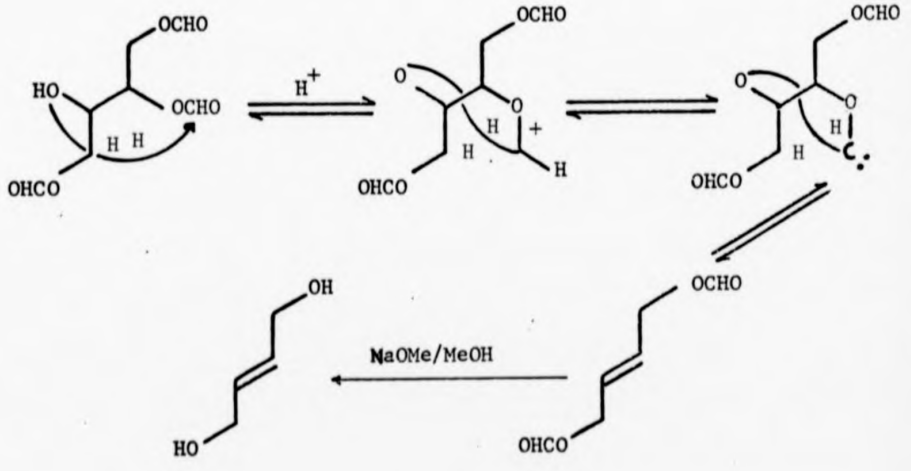
Supposed Mechanism for the Preparation of 1,2-Dihydroxybut-3-ene from Erythritol

SCHEME 4.2



Possible Mechanism of Formation of 1,4-dihydroxybut-2-ene

SCHEME 4.4



Mechanism for the stereoselective formation of *trans*-1,4-dihydroxybut-2-ene from the reaction of (2S,3S)- isopropylidene threitol with formic acid

The i.r. spectrum of both *cis*- and *trans*-isomers of 2-butene-1,4-diol have been reported[194] and are very different in the 8-14 μ region, with characteristic absorptions for each isomer. Closer examination of the by-product formed in the pyrolysis of the formate mixture from *meso*-erythritol by i.r. spectroscopy showed that it contained absorptions at 1085 and 1075 cm^{-1} characteristic of the *trans*-isomer. None of the *cis*-isomer could be detected. If the 2-butene-1,4-diol had been produced as a result of formylation of a secondary hydroxyl group in *meso*-erythritol, the proposed mechanism of formation of a double-bond from pyrolysis of formate esters (Scheme 4.3) predicts the production of a *trans*-double-bond. If this is correct then the use of (2R,3R)-(2,3-di-O)-isopropylidenethreitol should give *cis*-2-butene-1,4-diol as a by-product (Scheme 4.4).

4.3 SYNTHESIS OF 1,2-DIHYDROXYBUT-3-ENE

To facilitate the isolation of (2R,3R)-threitol, the hydroxyl groups of di-n-butyl tartrate were protected by an isopropylidene function. The tartrate diester was reacted with 2,2-dimethoxypropane to give the desired isopropylidene derivative in almost quantitative yield. Close inspection of the ^1H n.m.r. spectrum of this product revealed that 5% exchange of methoxy for butoxy groups had occurred. Even so, the (2R,3R)-(2,3-di-O)-isopropylidenethreitol obtained by reduction of (2R,3R)-di-n-butyl (2,3-di-O)-isopropylidenetartrate with lithium aluminium hydride was difficult to isolate from the precipitate formed during work up of the reaction mixture. To achieve good yields, the reaction had to be carried out on a fairly small scale and the solid material produced in the work up had to be extracted with ether in a Soxhlet apparatus.

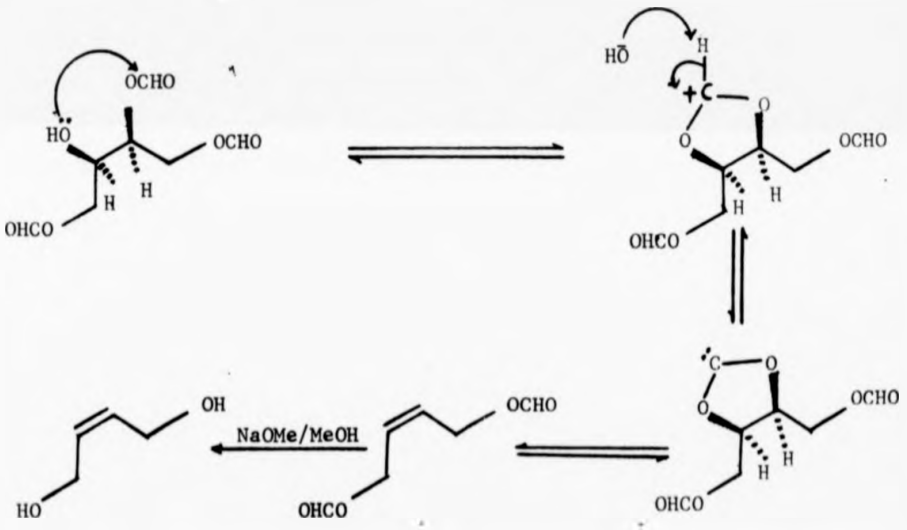
(2R,3R)-2,3-di-O-Isopropylidenethreitol was reacted with

formic acid in a similar manner to that used for *meso*-erythritol. There was no need to remove the isopropylidene group prior to the reaction because the acidic conditions ensured its rapid removal. As with *meso*-erythritol, distillation of the formic acid from the reaction mixture left a yellow-brown solid residue. Pyrolysis followed by hydrolysis yielded two products which co-distilled, but were separated by careful fractional distillation[195] with ethyl acetate as the solvent. ^{1}H n.m.r. spectroscopy indicated that the products were 3-butene-1,2-diol (R isomer: see below) and 2-butene-1,4-diol in approximately equal amounts. The i.r. spectrum of the latter compound was identical to that of commercial *cis*-2-butene-1,4-diol with a characteristic strong absorption of 1020 cm^{-1}. None of the *trans*-isomer could be detected. The ^{1}H n.m.r. spectrum of the *cis*- and *trans*-isomer of 2-butene-1,4-diol were almost identical. The fact that only *cis*-2-butene-1,4-diol is obtained from threitol, whereas *trans*-2-butene-1,4-diol arises from erythritol indicates that formylation of secondary hydroxyl groups does occur during the action of formic acid on either threitol or erythritol. These observations suggest that the 3-butene-1,2-diol obtained from (2R,3R)-threitol will be optically pure R isomer.

4.4 DETERMINATION OF THE OPTICAL PURITY OF (R)-3-BUTENE-1,2-DIOL

The specific rotation of the sample of (R)-3-butene-1,2-diol prepared from (2R,3R)-di-n-butyl tartrate, was measured as $[\alpha]_D^{25}$ = +40.04 (C = 4.62, propan-2-ol). This rotation was sufficiently lower than the value of $[\alpha]_D^{25}$ = -43.6 (C = 4.62, propan-2-ol) reported by Crawford[191] for there to be doubt about its optical purity. Before the diol could be used in the synthesis of (R)-lipoic acid, its optical purity had to be accurately determined.

SCHEME 4.3



Mechanism for the Stereoselective Fromation of *cis*-Dihydroxybut-2-ene from the Reaction of RS-erythritol with Formic Acid

Lanthanide shift reagents have been used successfully to determine the composition of enantiomeric mixtures. When suitable complexes of, e.g. europium and praseodynium become attached to a molecule they can have exceptionally marked effects on the chemical shifts of the protons of that molecule. If the ligand(s) of the lanthanide complex is itself an optically active molecule and consists of only one of the enantiomers then attachment to each isomer of an optically active molecule will produce diastereoisomeric complexes. Thus, the proton resonances of one enantiomer will be perturbed in a different way to those of the other enantiomer. The net result of this is that the n.m.r. spectrum of an enantiomeric mixture will show splitting of certain peaks into two, upon addition of an optically active shift reagent. The new peaks represent each enantiomer and the enantiomeric composition can be easily determined by integration.

A number of optically active lanthanide shift reagents were added to solutions of RS-1,2-dihydroxybut-3-ene in deuterochloroform but in all cases the ^{1}H n.m.r. spectrum was broadened too much and resolution was lost before any splitting of peaks occurred.

The presence of lanthanide oxide impurity in shift reagents causes excessive broadening of n.m.r. spectra. However, there was no improvement in the spectrum of (R)-1,2-dihydroxybut-3-ene even after the deuterochloroform, lanthanide solution was filtered to remove any oxide. Water in the shift reagent is often another cause of broadening of the n.m.r. spectrum, but again no improvement was incurred when the shift reagent was dried over phosphorus pentoxide in a vacuum desiccator. A different approach was obviously needed.

(R)-Ethyloxirane should be available by hydrogenation of (R)-1,2-dihydroxybut-3-ene and then conversion of the 1,2-diol function into an oxirane group. Assuming these reactions do not cause any racemisation, then determination of the enantiomeric composition

of the oxirane (by g.l.c. or ^{1}H n.m.r. with the aid of an optically active lanthanide complex) would give the optical purity of the (R)-1,2-dihydroxybut-3-ene.

An initial attempt to hydrogenate the double bond of (R)-1,2-dihydroxybut-3-ene using palladium (10% on barium sulphate) in methanol gave butane-1,2-diol contaminated with 20% of a by-product, identified by its ^{1}H n.m.r. spectrum as 1-hydroxy-2-oxobutane. This was presumed to have arisen by formation of a π-allyl complex between the palladium atom and (R)-1,2-dihydroxybut-3-ene resulting in an equilibrium between the palladium complex of 1,2-dihydroxybut-3-ene and 1,4-dihydroxybut-2-ene the latter isomer being easily converted to its oxo-form, 1-hydroxy-2-oxobutane. This is not a surprising result because palladium is well known to form π-allyl systems. Also, a similar isomerisation of allyl alcohol to propanal by [Ir (cod) L_2PF_6] L = PMe, Ph_2, has been reported[196,197].

A variety of other hydrogenation catalysts were used and the amount of ketone by-product was measured by integration of the ^{1}H n.m.r. spectrum of the product mixture. Wilkinson's catalyst (15% by weight) proved to be no better than the palladium catalyst, with 18.5% of ketone present in the product. Adam's catalyst (PtO_2) was used as 5% by weight and gave a product containing only 5% ketone by-product. It was necessary to find conditions by which pure (R)-butane-1,2-diol could be produced because if the formation of 1-hydroxy-2-oxobutane from 1,2-dihydroxybut-3-ene *via* a π-allyl intermediate were reversible then racemisation of the starting-material might occur. After further experimentation it was discovered that pre-hydrogenated Adam's catalyst (1% by weight) gave a quantitative yield of (R)-butane-1,2-diol in which no ketone could be detected either by ^{1}H n.m.r. or i.r. spectroscopy.

Treatment of (R)-butane-1,2-diol with 48% hydrogen bromide-acetic acid gave (R)-2-acetoxy-1-bromobutane. This was reacted with 1 mole equivalent of potassium pentyloxide in pentyl alcohol and (R)-ethyloxirane was distilled out of the reaction mixture at atmospheric pressure in 66% yield. The optical rotation of re-distilled (R)-ethyloxirane was measured as $[\alpha]_D^{25} = +11.6^\circ$ (C = 4.99, dioxane). The significantly higher literature value of $[\alpha]_D^{25} = -12.25$ (C = 6, dioxane)[198], confirms the suspicion that the (R)-1,2-dihydroxybut-3-ene contains a small amount of the S-isomer. The exact enantiomeric composition of the (R)-ethyloxirane and therefore (R)-1,2-dihydroxybut-3-ene was determined by means of an optically active lanthanide shift reagent.

Commercially available RS-ethyloxirane was used in initial experiments with optically active shift reagents to make sure that the ^1H n.m.r. spectrum of each enantiomer could be resolved and used as a reference for samples of unknown composition. Twenty-five milligrams of europium(III) *tris*[3-(heptafluorobutyl)-d-camphorate] was added to a solution of thirty milligrams of commercially available RS-ethyloxirane in 0.5 ml of deuterochloroform. In the resulting ^1H n.m.r. spectrum, a triplet of one proton of the oxirane ring methylene was split into two distinct triplets, separated by more than 0.1 p.p.m. Likewise, the multiplet due to the methine proton of RS-ethyloxirane was split into two completely separate multiplets.

A similar experiment was carried out using (R)-ethyloxirane derived from (R)-1,2-dihydroxybut-3-ene. Preliminary examination of the ^1H n.m.r. spectrum obtained after the addition of optically active shift reagent showed the presence of only one ring-methylene proton triplet. However, with scale expansion and high sensitivity a very small triplet in the correct position for the S-enantiomer of ethyloxirane was detected. The ratio of the two triplets was measured by weight because one of them was too small to be integrated. In this way the sample of

(R)-ethyloxirane was shown to contain 4.6% of the S-isomer.

4.5 POSSIBLE ORIGIN OF THE OPTICAL IMPURITY OF (R)-ETHYLOXIRANE

Partial racemisation could have occurred during the pyrolysis of the formate of (2R,3R)-2,3-di-O-isopropylidene reitol, or the hydrogenation of 1,2-dihydroxybut-3-ene, but this has been discounted in both cases. Had isomerisation occurred during pyrolysis, a mixture of *cis*- and *trans*-isomers of 1,4-dihydroxybut-2-ene would have been produced. However, pure *trans*-isomer was obtained from (2R,3R)-threitol and the pure *cis*—isomer from *meso*-erythritol. Racemisation of (R)-1,2-dihydroxybut-3-ene during hydrogenolysis could have been due to reversible formation of the ketone by-product. Because none of this was produced under the reaction conditions used, it can be safely assumed that racemisation did not occur in this way.

Perhaps the most likely explanation of the optical impurity in (R)-ethyloxirane is that it was present in the starting material of the synthesis, commercial (2R,3R)-di-n-butyl tartrate. This compound was used without purification or check of its optical purity.

4.6 FROM (R)-1,2-DIHYDROXYBUT-3-ENE TO (R)-LIPOIC ACID

Although the (R)-1,2-dihydroxybut-3-ene obtained from (2R,3R)-di-n-butyl tartrate was shown to be slightly optically impure, it was considered to be good enough to be used in experiments to establish the rest of the route to (R)-lipoic acid.

(S)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane is an intermediate in the synthesis of (S)-lipoic acid from (S)-malic acid. Its (R)-isomer is also readily synthesised from (R)-3-butene-1,2-diol. Conversion of (R)-3-butene-1,2-diol to (R)-4-(2'-hydroxy-

ethyl)-2,2-dimethyl-1,3-dioxolane was very straightforward. Firstly, (R)-2,2-dimethyl-4-vinyl-1,3-dioxolane was made by reaction of (R)-1,2-dihydroxybut-3-ene with acetone (in the presence of excess of anhydrous copper sulphate). After the mixture had been stirred at room temperature overnight a 74% yield of pure ketal was obtained. Hydroboration of the product, (R)-2,2-dimethyl-4-vinyl-1,3-dioxolane would enable water to be added across the double bond in an anti-Markovnikov fashion to give the desired (R)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane.

9-BBN is known to react with double bonds with very high regioselectivity[199]. Thus, when (R)-2,2-dimethyl-4-vinyl-1,3-dioxolane was reacted with 9-BBN in dry THF, and the trialkyl borane that formed was decomposed with sodium hydroxide and hydrogen peroxide, a 100% yield of 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane was obtained. This product was identical spectroscopically to (S)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane obtained from (S)-malic acid (see Chapter 5). As expected, the sign of optical rotation of each compound was opposite.

4.7 IMPROVEMENT OF THE SYNTHESIS

Work should be done to establish the optical purity of commercial tartrate diesters by means of optically active shift reagents or by accurate measurement of optical rotations. Because of the loss of two n-butyl groups in the reduction of (2R,3R)-di-n-butyl-(2,3-di-O)-isopropylidene tartrate a large decrease in molecular weight is incurred. This means that in order to obtain sizeable amounts of (2S,3S)-2,3-di-O-isopropylidene threitol an inconvenient, large weight of starting material is required. Also, work-up of the mixture from the reduction gave the desired product and n-butanol which has to be removed by fractional distillation. The use of (R,R)- or (S,S)-dimethyl tartrate as starting materials (also cheap and commercially

available) should be better.

Another problem with the large scale reduction of (2S,3S)-2,3-di-O-isopropylidene threitol was that a large amount of precipitate was obtained in the work-up procedure. This caused difficulty in the extraction of product with the small Soxhlet extractor available. To obtain the yields given in the Experimental section, the reduction was carried out in two separate experiments of relatively small scale. This problem could be solved by direct reduction of (2R,3R)-di-n-butyl tartrate with lithium aluminium hydride. Smith *et al.*[200] obtained threitol in 74% yield, using a special work-up procedure. Alternatively, (2R,3R)-dimethyl tartrate could be reduced with potassium borohydride in ethanol (reported yield[201] of (2R,3R)-threitol: 71%).

4.8 CONCLUSION

Even though the yield of 1,2-dihydroxybut-3-ene from (2R,3R)-di-n-butyl tartrate is greatly reduced by the formation of an equal amount of 1,4-dihydroxybut-2-ene, the overall yield of 40%, is the same as that of Crawford's synthesis[191] of (S)-1,2-dihydroxybut-3-ene from (S)-mannetol. Owing to the limitations with Crawford's synthesis, discussed in the Introduction to this Chapter, it was felt that the use of tartaric diesters as a starting material will be a superior method, when the small amount of optical impurity is removed.

Having shown that (R)-1,2-dihydroxybut-3-ene can be easily converted into (R)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane, work was concentrated on the remainder of the synthesis of lipoic acid. For this purpose (S)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane derived from (S)-malic acid was used and these results are discussed in Chapter 5.

EXPERIMENTAL

(R)-1,2-dihydroxybut-3-ene

(2S,3S)-(2,3-di-O)-isopropylidene threitol (27 g, 0.166 mol) and formic acid (98%, 65 ml) were placed in a 250 ml r.b. flask fitted with a downward condenser and receiving flask. The mixture was stirred with heating while the excess formic acid was distilled off at 108°. After 2 h, the distillation ceased and the flask was allowed to cool. The brown solid was heated in the flask with a naked flame and a distillate was collected between 140 and 230°. The product was distilled to give a colourless oil (13.9 g, 72%) b.p. 90-110°, 12 mmHg. This was taken up in dry methanol (50 ml) and sodium methoxide in methanol (7.8×10^{-3} M, 20 ml) was added. After the solution had been left at r.t. overnight, amberlite resin (IRC 50, 1.5 g) was added and the mixture stirred for 15 min. The solid material was filtered off and the solvent removed from the filtrate to yield a deep yellow oil which was distilled to give a colourless oil (7.38 g, 86.8%) b.p. 98-120, 15 mmHg.

T.l.c. indicated the presence of two products [ethyl acetate, 25% sulphuric acid with charring, produced brown spots on a white background, R_F 0.35 and 0.23]. Careful distillation of the product through a small, B-10 column packed with glass helices, enabled fractions to be collected of; (R)-1,2-dihydroxybut-3-ene b.p. 98-100°, 15 mmHg (2.06 g, 40%). $[\alpha]_D^{25}$ = +40.04 C = 4.62 propan-2-ol *cf.* lit.[191] $[\alpha]_D^{25}$ = -43.6 for S-isomer C = 4.62 propan-2-ol b.p. 58-60°, 2 mmHg.

I.r. (film): 3350 (brs), 3080 (w), 3005 (w), 2970 (w), 2920 (m), 2870 (m), 1420 (m), 1315 (w), 1130 (w), 1060 (s), 1020 (s), 920 (s), 860 (w).

N.m.r. ($CDCl_3$, TMS): 3.60 (m, 2 H, $-\underline{CH_2}OH$), 3.95 (br.s, 2 H, 2 x -OH),

4.26 (m, 1 H, -CHOH), 5.29 d of d's, 2 H, =CH_2), 5.83 (m, 1 H, =CH).

And *trans* -1,4-dihydroxybut-2-ene, b.p. 136-138°, 10 mmHg, (1.96 g, 38%).

I.r. (film): 3330 (brs), 2930 (m), 2870 (m), 1440 (brm), 1370 (m), 1220 (w), 1085 (s), 995 (s), 975 (s).

N.m.r. ($CDCl_3$, TMS): 4.12 (d J = 1 Hz, 4 H, 2 x -CH_2OH), 4.84 (brs, 2 H, 2 x -OH), 5.84 (d, J = 1 Hz, 2 H, HC=CH).

Lit. i.r.[194]: 3330 (brs), 2930 (m), 2875 (m), 1440 (brm), 1370 (m), 1210 (w), 1085 (s), 995 (s).

(2R,3R)-di-n-butyl (2,3-di-O)-isopropylidene tartrate

(2R,3R)-di-n-butyl tartrate (75 g, 0.29 mol) and 2,2-dimethoxypropane (62.4 g, 0.6 mol) were heated in benzene with p-toluenesulphonic acid (0.5 g), whilst the benzene-methanol azeotrope (b.p. 57°) was slowly distilled off at the head of a lagged Dufton column. The distillation was continued for 2 h until the temperature of the distillate reached 78°. The deep red solution was allowed to cool, then anhydrous potassium carbonate (1.5 g) was added and the mixture stirred for 10 min. The solid material was filtered off and the solvent removed to yield a red oil. Distillation of the crude product gave a colourless oil (85.25 g, 98.7%) b.p. 148-150°, 3 mmHg, $[\alpha]_D^{25}$ = -31.4 (C = 5, chloroform)

I.r. (film): 3960 (s), 3940 (m), 3880 (m), 1720 (v.s), 1465 (m), 1385 (m), 1375 (m), 1260 (m), 1210 (s), 1110 (s), 1070 (m), 1020 (w), 970 (w), 870 (w), 740 (w).

N.m.r. (CCl_4, TMS): 0.98 (t, 6 H, 2 x CH_3), 1.46 (s and m, 10 H, 2 x CH_2CH_3 and -$C(CH_3)_2$), 1.68 (p, 4 H, 2 x -OCH_2CH_2-), 4.16 (t, 4 H, 2 x -OCH_2), 4.69 (s, 2 H, 2 x HC-O-).

(2S,3S)-(2,3-di-O)-isopropylidenethreitol

To a suspension of lithium aluminium hydride (7.88 g, 0.21 mol) in dry ether (100 ml) was added a solution of (2S,3S)-di-n-butyl (2,3-di-O)-

isopropylidene tartrate (32.15 g, 0.105 mol) in dry ether (100 ml) dropwise with stirring over a period of 1½ h. When the addition was complete, the mixture was heated at reflux for 3 h and then allowed to cool to r.t. Further cooling in ice and careful successive addition of water (8 ml), 15% sodium hydroxide solution (8 ml) and water (24 ml), with stirring resulted in the formation of a white granular substance. The precipitate was filtered off, washed with ether and then extracted in a Soxhlet apparatus with ether, overnight. All organic fractions were combined, dried (anhydrous magnesium sulphate) and evaporated to give a pale yellow oil. The crude product was purified by fractional distillation to give two constant boiling fractions of: butan-1-ol b.p. 117°, 760 mmHg. and (2S,3S)-(2,3-di-O)-isopropylidene threitol b.p. 98-100°, 0.25 mmHg (15.56 g, 90.5%) $[\alpha]_D^{25} = +6^\circ$ [C = 5, $CHCl_3$].

I.r. (film): 3400 (br.s), 2980 (m), 2940 (m), 2880 (m), 1460 (w), 1385 (s), 1375 (s), 1255 (s), 1220 (s), 1165 (m), 1110 (m), 1060 (s), 990 (w), 900 (w), 880 (w), 845 (m), 800 (w).

N.m.r. ($CDCl_3$, TMS): 1.43 (s, 6 H, $-CMe_2$), 2.28 (br.s, 2 H, 2 x $-OH$), 3.76 (m, 2 x CH_2OH), 4.10 (br.s, 2 H, 2 x $-CH$).

The preparation was carried out once more in similar yields to give a further 16.2 g of product which was combined.

RS-1,2-Dihydroxybut-3-ene

Meso-erythritol (21 g, 0.17 mol) and formic acid (80 ml, 98-100%) were heated with stirring while the excess formic acid was distilled off at 80-108°. After 2½ h the distillation ceased, to give a brown oil which was pumped at 0.001 mmHg with gentle heating (50° water bath) for 2 h to remove traces of formic acid. Upon cooling a yellow brown solid of di- and tri-formates was

obtained which was gently heated with a naked flame. The solid melted and carbon dioxide began to be given off. Continued heating resulted in a distillate being collected at 140°. The oil was slowly distilled with a naked flame whilst the temperature of the distillate rose gradually to 230°. The oil that was collected was redistilled to give a colourless oil (9.05 g, 85%) b.p. 90-110°, 14 mmHg. An ^{1}H n.m.r. spectrum showed the product to consist of a mixture of 1,2-dihydroxybut-3-ene 1-O-formate:

($CDCl_3$, TMS) 2.69 (brs, 1 H, OH), 4.26 (m, 2 H, $-CH_2OCHO$), 5.37 (m, 2 H, $-CH_2-CH$), 5.82 (m, 1 H, $-CH=CH_2$), 8.13 (s, 1 H, -OCHO).

And 2-butene-1,4-diol 1-O-formate:

($CDCl_3$, TMS) 4.70 (d, 4 H, 2 equivalent -CH's), 5.92 (s, 2 H, 2 equivalent HC=C's).

The crude product (13.74 g) was hydrolysed with sodium methoxide in methanol to give a colourless oil (5.15 g, 91.6%) of crude product. Pure (R)-3-butene-1,2-diol was obtained by flash column chromatography[195].

T.l.c. of mixture before purification [ethyl acetate, spots charred with sulphuric acid, R_F 0.34 ((R)-1,2-dihydroxybut-3-ene and R_F 0.26 (*cis*-1,2-dihydroxybut-2-ene)].

A 55 mm diameter chromatography column was packed six inches deep with Kieselgel 60. Crude product (2.5 g) was loaded onto the column and eluted with ethyl acetate. Fractions (36 x 50 ml) were collected with a flow rate of 2" per minute.

Fractions 1-14 gave an oil (1.1 g, 44% recovery) of pure RS-1,2-dihydroxybut-3-ene. The ^{1}H n.m.r. spectrum was identical to that of (R)- 1,2-dihydroxybut-3-ene.

Evaporation of fractions 16.36 yielded an oil (1.17 g, 47%,

R_F 0.23) of pure *cis*- 1,4-dihydroxybut-2-ene, b.p. 135°, 10 mmHg.

I.r. (film): 3320 (brs), 3020 (w), 2930 (w), 2880 (w), 1420 (brw), 1330 (brw), 1240 (brw), 1210 (brw), 1020 (s), 970 (m), 940 (w).

N.m.r. ($CDCl_3$, TMS): 4.09 (s, 4 H, 2 x $-CH_2OH$), 4.56 (brs), 2 H, 2 x OH), 5.82 (s, 2 H, HC=CH).

Cf. i.r. commercial *cis*- 1,4-dihydroxybut-2-ene (film): 3320 (brs), 3020 (w), 2930 (w), 1425 (w), 1330 (brw), 1240 (brw), 1210 (brw), 1020 (s), 970 (m), 940 (w).

N.m.r. commercial *cis*- 1,4-dihydroxybut-2-ene ($CDCl_3$, TMS): 4.08 (s, 4 H), 2 x CH_2OH), 4.50 (brs, 2 H, 2 x OH), 5.82 (s, 2 H), HC=CH).

(R)-Butane-1,2-diol: General procedure for the hydrogenation of (R)-3-butene-1,2-diol

Hydrogenation catalyst was placed in a 2-necked, 25 ml r.b. flask, fitted with gas inlet and suba seal and containing a magnetic follower and methanol (10 ml). The flask was put under hydrogen at atmospheric pressure and

a solution of (R)-1,2-dihydroxybut-3-ene (1.42 g, 0.016 mol) in methanol (5 ml) was syringed into the mixture which was then stirred overnight, by which time hydrogen uptake had ceased. The catalyst was carefully filtered off and the solvent evaporated to give a product which was examined by ^1H n.m.r. spectroscopy. Depending on the catalyst used, the product consisted of varying ratios of (R)-butane-1,2-diol, b.p. 70-72°, 10 mmHg.

N.m.r. ($CDCl_3$, TMS): 0.95 (t, 3 H, $-CH_3$), 1.45 (q, 2 H, $-CH_2CH_3$), 3.41 (brt, 1 H, HCOH), 3.63 (br.d, 2 H, $-CH_2OH$), 3.74 (br.s, 2 H, 2 x -OH).

And 1-hydroxy-2-oxobutane:

N.m.r. ($CDCl_3$, TMS): 1.13 (t, 3 H, $-CH_3$), 2.45 (q, 2 H, $-CH_2CH_3$), 3.83 (br.s, 1 H, -OH), 4.28 (s, 2 H, $HOCH_2CO$). For results see Table 4.1.

R_F 0.23) of pure *cis*- 1,4-dihydroxybut-2-ene, b.p. 135°, 10 mmHg.

I.r. (film): 3320 (brs), 3020 (w), 2930 (w), 2880 (w), 1420 (brw), 1330 (brw), 1240 (brw), 1210 (brw), 1020 (s), 970 (m), 940 (w).

N.m.r. ($CDCl_3$, TMS): 4.09 (s, 4 H, 2 x $-\underline{CH_2}OH$), 4.56 (brs), 2 H, 2 x $O\underline{H}$), 5.82 (s, 2 H, $\underline{HC}=\underline{CH}$).

Cf. i.r. commercial *cis*- 1,4-dihydroxybut-2-ene (film): 3320 (brs), 3020 (w), 2930 (w), 1425 (w), 1330 (brw), 1240 (brw), 1210 (brw), 1020 (s), 970 (m), 940 (w).

N.m.r. commercial *cis*- 1,4-dihydroxybut-2-ene ($CDCl_3$, TMS): 4.08 (s, 4 H), 2 x $C\underline{H_2}OH$), 4.50 (brs, 2 H, 2 x $O\underline{H}$), 5.82 (s, 2 H), $\underline{HC}=\underline{CH}$).

(R)-Butane-1,2-diol: General procedure for the hydrogenation of (R)-3-butene-1,2-diol

Hydrogenation catalyst was placed in a 2-necked, 25 ml r.b. flask, fitted with gas inlet and suba seal and containing a magnetic follower and methanol (10 ml). The flask was put under hydrogen at atmospheric pressure and

a solution of (R)-1,2-dihydroxybut-3-ene (1.42 g, 0.016 mol) in methanol (5 ml) was syringed into the mixture which was then stirred overnight, by which time hydrogen uptake had ceased. The catalyst was carefully filtered off and the solvent evaporated to give a product which was examined by ^1H n.m.r. spectroscopy. Depending on the catalyst used, the product consisted of varying ratios of (R)-butane-1,2-diol, b.p. 70-72°, 10 mmHg.

N.m.r. ($CDCl_3$, TMS): 0.95 (t, 3 H, $-\underline{CH_3}$), 1.45 (q, 2 H, $-\underline{CH_2}CH_3$), 3.41 (bzt, 1 H, $\underline{H}COH$), 3.63 (br.d, 2 H, $-\underline{CH_2}OH$), 3.74 (br.s, 2 H, 2 x $-O\underline{H}$).

And 1-hydroxy-2-oxobutane:

N.m.r. ($CDCl_3$, TMS): 1.13 (t, 3 H, $-\underline{CH_3}$), 2.45 (q, 2 H, $-\underline{CH_2}CH_3$), 3.83 (br.s, 1 H, $-O\underline{H}$), 4.28 (s, 2 H, $HOC\underline{H_2}CO$). For results see Table 4.1.

TABLE 4.1

Amount of 1-Hydroxy-2-oxobutane By-product formed during the Reduction of (S)-3-Butene-1,2-diol to Butane-1,2-diol using Different Catalysts

Catalyst	% Weight	% By-product
Pd (10% on $BaSO_4$)	10	20
Wilkinsons	15	18.5
Adams	5	5
Adams (prehydrogenated)	1	0

(R)-2-Acetoxy-1-bromobutane

(R)-Butane-1,2-diol (1.32 g, 14.6 mmol) was dissolved in 48% w/v hydrogen bromide-acetic acid (6.9 ml, 43.8 mmol of HBr). The mixture was protected from moisture and stirred for 30 min. when water (25 ml) was added and the acid neutralised quickly with solid potassium carbonate. Extraction of the mixture with ether (3 x 25 ml) gave, after the organic phase was dried (anhydrous potassium carbonate) and the solvent removed, a yellow oil. The crude product was distilled on a Kugelröhr apparatus to give a colourless oil (2.02 g, 88%).
N.m.r. (CCl_4, TMS): 0.94 (t, 3 H, $-CH_2\underline{CH_3}$), 1.70 (m, 2 H, $-\underline{CH_2}CH_3$), 2.04 (s, 3 H, $\underline{CH_3}CO$), 7.41 (t, 2 H, $-\underline{CH_2}Br$), 4.82 (p, 1 H, Ac$\underline{CH}$).
< 5% of (2R)-1-acetoxy-2-bromobutane was detected in the n.m.r. spectrum of the product.

(R)-Ethyloxirane

A solution of (R)-2-acetoxy-1-bromobutane (2.0 g, 0.01 mol) in dry pentyl alcohol (4.4 ml) was placed in a 25 ml, B-10 pear shaped flask and a 0.821 M solution of pentyl oxide in pentyl alcohol (12.17 ml, 0.0099 mol) was added. The reaction mixture was stirred and heated (oil bath 140°) while a distillate boiling at 70-90° was collected in a cold trap (acetone-dry ice). The colourless oil (0.73 g, 100%) was purified by a trap to trap distillation to give a volatile produce (0.483 g, 66%) b.p. 63-65°, 760 mmHg, $[\alpha]_D^{25}$ = +11.6 (C = 4.99, dioxane, *cf.* lit.[198] $[\alpha]_D^{25}$ = -12.25 (C = 6, dioxane) for S-isomer b.p. 64-65°.
N.m.r. ($CDCl_3$, TMS): 1.01 (t, 3 H, $-\underline{CH_3}$), 1.58 (m, 2 H, $-\underline{CH_2}CH_3$), 2.49 (m, 1 H, 1'H of ring methylene), 2.74 (t, 1 H, 1'H of ring methylene), 2.89 (m, 1 H, ring methine).

2,2-Dimethyl-4-vinyl-1,3-dioxolane

(R)-1,2-dihydroxybut-3-ene (1.3 g, 1.47 x 10^{-2} mol) was dissolved in acetone

(10 ml) and anhydrous copper sulphate (6.5 g) was added. The mixture was stirred overnight at room temperature and then the solid material was filtered off and the filtrate fractionally distilled to give a fraction of acetone, b.p. 56°, 760 mmHg and a fraction of pure 2,2-dimethyl-4-vinyl-1,3-dioxolane (1.40 g, 74.2%), b.p. 85°, 125 mmHg.

N.m.r. (CCl_4, TMS): 1.32 (s, 3 H, and 1.36 s, 3 H, $-CMe_2$), 3.47 (t, 1 H, and 3.98 t, 1 H, $-CH_2$ of dioxolane ring), 4.46 (q, 1 H, -CH), 5.19 d of d's, 2 H, $=CH_2$), 5.76 (m, 1 H, =CH). $[\alpha]_D^{25}$ = -25.50 (C = 9.8, propan-2-ol) *cf.* lit.[191] +30.96° (C = 9.8, propan-2-ol).

4-(2'-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolane

2,2-Dimethyl-4-vinyl-1,3-dioxolane (0.33 g, 2.5 mmoles) was weighed into a 25 ml 3-necked B-10 flask fitted with dropping funnel, stopper and air condenser. The apparatus was protected from moisture and dry THF was added (2 ml). A solution of 9-BBN (0.3 g, 2.5 mmol) in dry THF (3 ml) was added over 10 min. and then the mixture was stirred for 2 h at r.t. After this period of time sodium hydroxide solution (3 M, 0.83 ml) was carefully added with stirring followed by dropwise addition of hydrogen peroxide (30%, 0.83 ml). Ether (25 ml) was added to the reaction mixture and the immiscible aqueous layer separated and extracted with more ether (3 x 20 ml). The organic fractions were combined, dried (anhydrous magnesium sulphate) and evaporated to give a colourless oil of crude product which was distilled in a Kugelröhr apparatus (10 mmHg, oven temp. 135-145°). The resulting colourless oil of 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane, b.p. 98-100°, 10 mmHg, $[\alpha]_D^{25}$ = +1.3 (C = 4.6, methanol) lit. $[\alpha]_D^{25}$ = 1.29° (C = 4.6, methanol) was pure by:

I.r. (film: 3430 (brs), 2990, 2940 (s), 2980 (s), 1455 (w), 1380 (s), 1370 (s), 1250 (s), 1220 (s), 1160 (s), 1060 (s), 990 (w), 960 (w),

940 (w), 860 (m), 820 (w), 795 (w).

N.m.r. ($CDCl_3$, TMS): 1.38 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 1.86 (m, 2 H, J = 5 Hz), 3.60 and 4.10 (t, 1 H, ring $-CH_2$), 3.79 (t and br.s, 3 H, CH_2OH, J = 6 Hz, and CH_2OH), 4.37 (p, 1 H, ring methine).

Optically active shift reagent experiments on (R)-Ethyloxirane

RS-Ethyloxirane (30 mg) was dissolved in deuterochloroform (0.5 ml) and the solution transferred to an n.m.r. tube. The lanthanide shift reagent, europium heptafluorobutylcamphorate was added in successive 5 mg amounts, the ^1H n.m.r. spectra being taken between additions. The results are shown in Table 4.2.

(R)-Ethyloxirane (30 mg) was dissolved in deuterochloroform (0.5 ml) and the solution transferred to an ^1H n.m.r. tube. Europium(III)-*tris*[3-(heptafluorobutyl)-d-camphorate] was added to the tube and the ^1H n.m.r.spectrum taken. The spectrum showed only one triplet at δ3.54 and one multiplet at δ3.64 with no sign of splitting. Upon scale expansion and increase in sensitivity a very small triplet appeared at δ3.56 - the position of the triplets of (S)-isomer in RS-ethyloxirane. Thus, it was estimated by weight that the sample contained 4.6% of (S)-ethyloxirane impurity.

TABLE 4.2

Effect on the ^{1}H n.m.r. Spectrum of *Rac*-ethyloxirane upon the Addition of Europium(III)Tris 3-(Heptafluorobutylcamphorate)

OMg shift reagent	5 mg	10 mg	15 mg	20 mg	25 mg
m @ 2.89	m @ 3.17	m @ 3.42	m @ 3.60	m @ 3.87	2m @ 4.16 & 4.25
t @ 2.74	q @ 2.76	2t @ 3.22	2t @ 3.34 & 3.41	2t @ 3.58 & 367	2t @ 3.88 & 3.98

CHAPTER 4 - REFERENCES

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CHAPTER 5

SYNTHESIS OF (S)-LIPOIC ACID FROM (S)-MALIC ACID

5.1 INTRODUCTION

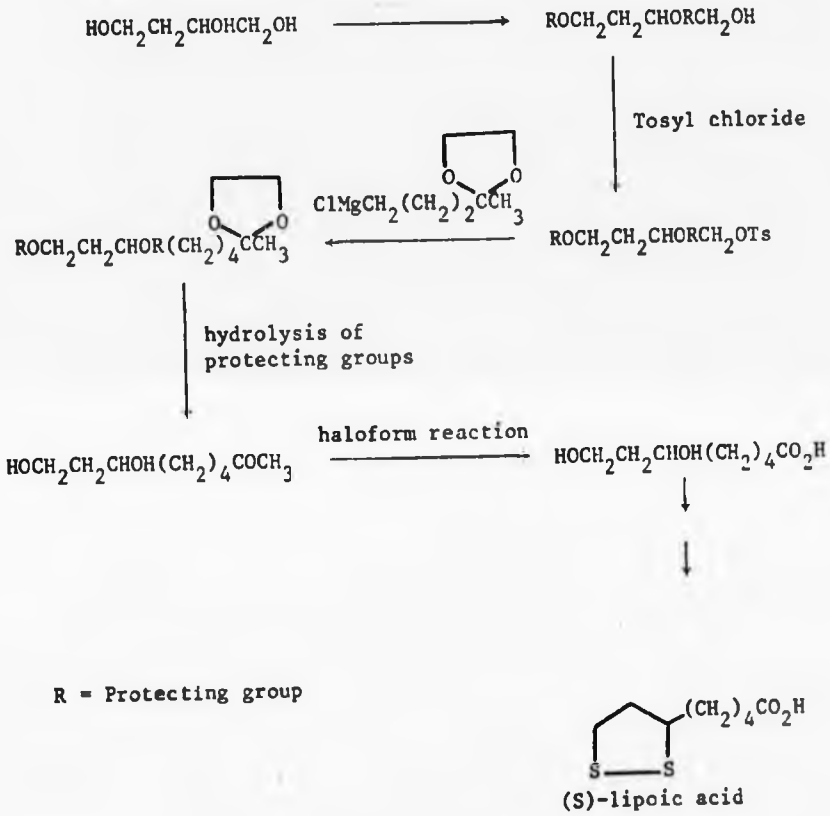
During the course of the work on the synthesis of (S)-lipoic acid from (S)-malic acid *via* (S)-butane-1,2,4-triol (as discussed in Chapter 2), a second route using the triol intermediate was developed. Protection of the 2- and 4-hydroxy groups of (S)-butane-1,2,4-triol would enable the terminal 1-hydroxy group to be tosylated. Direct displacement of the tosylate with a Grignard reagent from 2-methyl-2-(3'-chloropropyl)-1,3-dioxolane would then produce the eight carbon atom backbone of lipoic acid. Hydrolysis of the hydroxy and keto-protecting groups would yield (S)-7,9-dihydroxy-2-oxononane, an intermediate in the original route and easily convertible to (S)-lipoic acid (Scheme 5.1). Therefore, this Chapter discusses the synthesis of (S)-butane-1,2,4-triol and then outlines the work done on the two different routes available to (S)-lipoic acid.

5.2 SYNTHESIS OF (S)-BUTANE-1,2,4-TRIOL FROM MALIC ACID

(S)-Malic acid was converted to its diethyl ester to facilitate its reduction. The diester was prepared by the azeotropic mixture method[202] in almost quantitative yield. The procedure gave a much higher yield of product than the Fischer method of esterification previously employed for the preparation of (S)-diethyl malate acid.

(S)-Diethyl malate was reduced to butane-1,2,4-triol with lithium aluminium hydride in 35% yield. The lower yield than was expected for a reduction of this kind, was a consequence of the extremely polar nature of the triol, causing it to bind to the

SCHEME 5.1



R = Protecting group

(S)-lipoic acid

An alternative route to (S)-lipoic acid using (S)-butane-1,2,4-triol

precipitate of aluminates formed during work up of the reaction mixture. In order to avoid this problem a number of attempts were made to protect the hydroxyl group of (S)-diethyl malate. Reduction of the derivative would then lead to a product containing only two hydroxyl groups which should be easier to isolate. Removal of the protecting group would then afford the desired triol.

Corey *et al.*[203] reacted (S)-diethyl malate with 2-methoxypropene, under catalysis by phosphorus oxychloride, at 23° for 1 hour and obtained a quantitative yield of protected ester. This underwent reduction with lithium aluminium hydride in 79% yield to give 2-(2'-methoxypropyl)-butane-1,4-diol. This product seemed a particularly attractive compound because on treatment with boron trifluoride diethyl etherate in ether at 2° for 2 hours, 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane was obtained in 86% yield[203]. This is an intermediate in the proposed route to (S)-lipoic acid form. (S)-Malic acid and its synthesis by Corey's route[203] would avoid the necessity of having to prepare butane-1,2,4-triol.

2-Methoxypropene was prepared from 2,2-dimethoxypropane by the method of Newman and Vander Zwan[204]. Corey's[203] preparation of 2-methoxyprop-2-yl ether of (S)-diethyl malate was followed. They had used conditions employed by Kluge *et al.*[205]. Unfortunately the procedure could not be reproduced. Instead of obtaining a 100% yield of product, with no purification step needed, a black residue was obtained which consisted of mainly unreacted starting material. The reaction conditions were modified in an effort to get the reaction to proceed satisfactorily. The use of 2-methoxypropene as solvent for the reaction seemed undesirable because it was possible for it to undergo intermolecular reaction with itself before reacting with (S)-diethyl malate. Thus, a slight excess of 2-methoxypropene was stirred with

(S)-diethyl malate in chloroform overnight. However, work up of the reaction mixture gave a black oil containing chiefly unreacted starting material.

Tetrahydropyranyl is a convenient protecting group for alcoholic functions[206,207], but suffers from the disadvantage that a new asymmetric centre is introduced into the protected molecule. Shealy[208] *et al.* have reported the synthesis of (S)-diethyl-2-O-(2'-tetrahydropyranyl)malate and its reduction, with lithium aluminium hydride, to (S)-2-O-(2'-tetrahydropyranyl)-butane-1,2,4-triol. However, these reactions took place in yields of 37.5% and 47% respectively.

(S)-Butane-1,2,4-triol was finally obtained by the procedure of Nakanishi *et al.*[209]. Diethyl malate was reduced with lithium aluminium hydride and the precipitate produced during work up was washed with a large amount of methanol. This procedure extracted a quantity of triol and some inorganic material, which was effectively removed by short column chromatography. In this way, a 58% yield of pure (S)-butane-1,2,4-triol was obtained.

5.3 LIPOIC ACID FROM (2 -BENZYLOXYETHYL)OXIRANE

(S)-(-)-4-(2'-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolane was prepared by acid-catalysed reaction of butane-1,2,4-triol with acetone. The ^{1}H n.m.r. data were consistent with the formation of a 1,3-dioxolane ring (i.e. protection of the vicinal hydroxyl groups). The coupling patterns of the signals at δ 1.83 (dt, 2 H, J = 5.5 and 6 Hz, $HOCH_2\underline{CH_2}$-) and 3.81 (t, 2 H, J = 6 Hz, $\underline{CH_2}OH$) denote the grouping $HOCH_2CH_2CH$ and are compatible with the 1,3-dioxolane structure, but not with the isomeric 4-hydroxymethyl-1,3-dioxane.

Golding and Ioannou[210] reported a rapid means of protecting a terminal hydroxy group of the triol glycerol. Glycerol was

converted to 4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane and this compound was benzylated by a phase transfer-catalysed reaction with benzyl chloride. Hydrolysis of the isopropylidene group gave 3-benzyloxypropane-1,2-diol.

4-(2'-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolane was benzylated in a two-phase system consisting of excess benzyl chloride, aqueous sodium hydroxide and a catalytic amount of benzyl tri-n-butylammonium bromide. The product from this reaction was not purified but subjected to immediate acidic hydrolysis. This removed the isopropylidene group and produced 4-benzyloxybutane-1,2-diol which could be easily isolated in excellent yield. The redistilled product was pure by ^{1}H n.m.r. spectroscopy, i.r. spectroscopy, and t.l.c. The overall yield from 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane was 87%.

An attempt to synthesise (2 -benzyloxyethyl)oxirane from 4-benzyloxybutane-1,2-diol by the method of Golding *et al.*[133] failed because the hydrogen bromide/acetic acid (HBA) reagent proved to be an effective debenzylating agent.

In 1980, Schurig *et al.*[212] proved that (2S,3S)-dimethyloxirane obtained by the method of Seeley and McElwe[213] was at least 99.9% diastereomerically and enantiomerically pure. The method of oxirane formation in ref. 213 should not affect the benzyl group. Thus, 4-benzyloxybutane-1,2-diol was refluxed with benzaldehyde and catalytic p-toluenesulphonic acid in benzene with azeotropic removal of water, to give 4-(2'-benzyloxyethyl)-2-phenyl-1,3-dioxolane in quantitative yield. Although this product was slightly discoloured it was pure by ^{1}H n.m.r. spectroscopy and t.l.c. and therefore used without further purification. The ^{1}H n.m.r. spectrum showed singlets at δ 5.77 and 5.88 indicating an epimeric mixture. These epimers were present in a ratio of 1:1.4. The dioxolane ring was opened by reaction with N-bromosuccinimide to give

pure (S)-2-benzyloxy-4-benzyloxy-1-bromobutane in quantitative yield. The oily product was slightly yellow but was pure by t.l.c. and ^1H n.m.r. spectroscopy and was used directly. (S)-(2 -Benzyloxyethyl)oxirane was obtained by treatment of (S)-2-benzyloxy-4-benzyloxy-1-bromobutane in ethane-1,2-diol with 2 mole equivalents of sodium hydroxide. Extraction of the mixture with petroleum ether gave, after evaporation of solvent, the crude oxirane derivative which was fractionally distilled to give a 70% yield of pure product.

5.4 . DETERMINATION OF THE ENANTIOMERIC PURITY OF (S)-(2-BENZYLOXYETHYL)OXIRANE

The optical purity of (S)-(2 -benzyloxyethyl)oxirane was determined with the aid of the optically active shift reagent europium(III) *tris*[3-(heptafluorobutyl)-d-camphorate]. Experiments were first performed on racemic material synthesised from racemic malic acid. This technique indicated an optical purity of ≥ 98%.

5.5 COUPLING OF (S)-(2 -BENZYLOXYLETHYL)OXIRANE WITH 2-(3'-CHLOROPROPYL)-2-METHYL-1,3-DIOXOLANE

Grignard reagents have been successfully reacted with oxirane derivatives, preferably in the presence of lithium tetrachlorocuprate as catalyst[214]. The Grignard reagent from 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane was prepared in THF and was reacted with (S)-(2 -benzyloxyethyl)oxirane. Purification of crude product by column chromatography gave pure 2-(7'-benzyloxy-5'-hydroxyheptyl)-2-methyl-1,3-dioxolane which was benzylated. This was done primarily to avoid any oxidation of the hydroxy group in the subsequent hypobromite oxidation.

Accordingly, 2-(7'-benzyloxy-5'-hydroxyheptyl)-2-methyl-1,3-dioxolane was converted to 2-(5',7'-dibenzyloxyheptyl)-2-methyl-1,3-dioxolane by treatment with excess benzyl chloride, aqueous sodium hydroxide and

phase transfer catalyst. Hydrolysis of the crude product with dilute sulphuric acid gave a mixture of 9-benzyloxy-7-hydroxy-2-oxononane and 7,9-dibenzyloxy-2-oxononane. After column chromatography of the crude product, 7,9-dibenzyloxy-2-oxononane containing 30% dibenzyl ether was obtained in 28% yield and pure 9-benzyloxy-7-hydroxy-2-oxononane in 37% yield.

There was insufficient time available to evaluate properly further steps in the synthesis. A preliminary haloform reaction applied to the mixture of 7,9-dibenzyloxy-2-oxononane and dibenzyl ether was promising (spectroscopic evidence for the production of a carboxylic acid).

5.6 AN ALTERNATIVE ROUTE TO (S)-LIPOIC ACID

(a) Synthesis of 4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate

The conversion of 4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane to 4-hydroxymethyl-2,2-dimethyl-1,3-dioxane was attempted but exposure of the dioxolane to acidic catalysis did not cause detectable isomerisation to the dioxane.

Foster *et al.*[215] studied the acid-catalysed reaction between RS-butane-1,2,4-triol and benzaldehyde. The product was shown to consist of 90-95% 4-hydroxymethyl-2-phenyl-1,3-dioxane and 5-10% 2-phenyl-4-(2'-hydroxyethyl)-2-phenyl-1,3-dioxolane. Pure 4-hydroxymethyl-2-phenyl-1,3-dioxane was obtained from this mixture *via* p-phenylazobenzoate derivatives. This work provided a means of protecting the 2,4-hydroxy groups of butane-1,2,4-triol.

(S)-Butane-1,2,4-triol, benzaldehyde and catalytic p-toluenesulphonic acid were refluxed in benzene with azeotropic removal of water. Work up of the reaction mixture afforded a crude product which was distilled. The ^{1}H n.m.r. data of the resulting product revealed that it contained predominantly 4-hydroxymethyl-2-phenyl-1,3-dioxane.

A doublet at δ 3.11 is assigned to the methylene protons, coupled to the adjacent ring proton (H-4), of the hydroxymethyl group at C-4. The hydroxyl group is a broad singlet at δ 2.90. A sharp singlet at δ 5.50 originates from H-2. The C-6 methylene protons give a multiplet at δ 3.93. Complex signals at δ 1.40 and 1.85 are assigned to $H\text{-}5_{ax}$ and $H\text{-}5_{eq}$, respectively. A small triplet at δ 3.72 indicates the presence in the $-CH_2\underline{CH}_2OH$ moiety of the isomeric C-membered ring product. Singlets at δ 5.77 and 5.90 are attributed to the *cis*-and *trans* forms of 4(1-hydroxyethyl)-2-phenyl-1,3-dioxolane.

The amounts of these compounds, in the acetal mixture, measured by integration of the benzylidene peaks, was found to be 6% for each of the *cis*- and *trans*-isomers. The fact that only the *cis*-isomers of 4-hydroxy methyl-2-phenyl-1,3-dioxolane was formed was due to the ability of ring substituents to adopt equatorial positions in a chair conformation for this isomer.

The mixture of acetals was tosylated by a standard method[188]. Pure 4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate was obtained by two recrystallisations of the solid material obtained from crysallisation of the oily product.

(b) **Reaction of 4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate with 2-methyl-2-(3'-chloropropyl)-1,3-dioxolane**

The Grignard reagent was made from 2-methyl-2-(3'-chloropropyl)-1,3-dioxolane in the usual manner, but was found not to react with

4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate, even after overnight boiling at reflux. The lack of reactivity of the tosylate may be due to steric hindrance at the carbon atom bearing the tosylate group.

4-Hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate was refluxed in methanol containing base. It was hoped that the tosyl group would be displaced by the neighbouring oxygen atom, leading to a useful optically active oxirane (see Scheme 5.2). However, no reaction of any kind occurred under these conditions and the use of 4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate was abandoned.

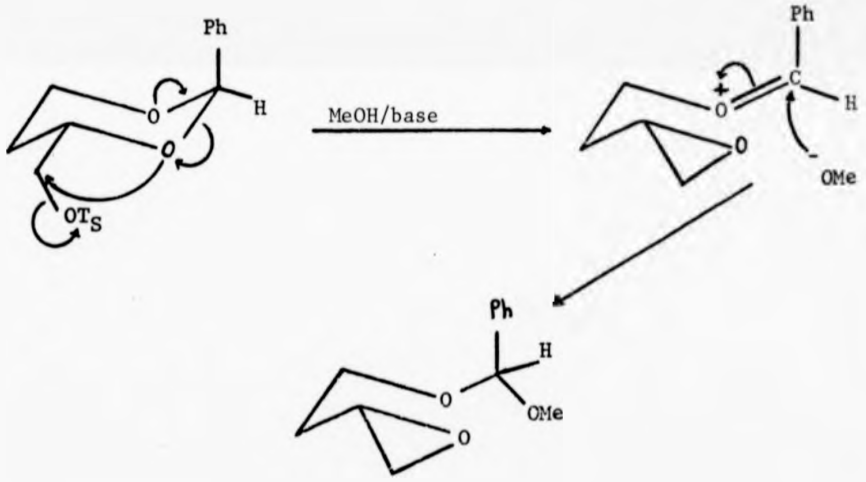
5.7 ATTEMPTED PREPARATION OF (2-ACETOXYETHYL)OXIRANE

An attempt was made to form the oxirane derivative of (S)-butane-1,2-triol without the need for prior protection of the 4-hydroxyl group. The triol was reacted with hydrogen bromide in acetic acid to give pure (S)-2,4-diacetoxy-1-bromobutane in 80% yield. It was hoped that treatment of this bromide with base would afford (2-acetoxyethyl)-oxirane, but in practice a mixture of unidentified products were obtained.

5.8 CONCLUSION

The failure of 4-hydroxymethyl-2-phenyl-1,3-dioxane p-toluene-sulphonate to react with the Grignard reagent from 2-methyl-2-(3'-chloropropyl)-1,3-dioxolane was disappointing but not disasterous because work on the alternative route from (S)-malic acid to (S)-lipoic was progressing well. The important intermediate, (2 -benzyloxyethyl)oxirane was synthesised from (S)-malic acid in seven straightforward steps in an overall yield of 25%. The oxirane derivative was found to be optically pure by experiments with optically active shift reagent and it reacted satisfactorily with the

SCHEME 5.2



Possible Formation of a useful Oxirane by Treatment of 4-Hydromethyl-2-phenyl-1,3-dioxolane p-toluenesulphonate with methanol/base

Grignard reagent from 2-methyl-2-(3'-chloropropyl)-1,3-dioxolane. The successful coupling of C_4-fragments was a very important result because it produced the lipoic acid skeleton in optically pure form.

Future work is likely to entail improving the yield of 7,9-dibenzyloxy-2-oxononane, optimisation of the conditions of the haloform reaction and the completion of the synthesis of (S)-lipoic acid from 7,9-dibenzyloxyoctanoic acid using reactions which have been used in the syntheses of RS-lipoic acid (see Chapter 2).

EXPERIMENTAL

S-(-)-Diethyl malate

(S)-Malic acid (83 g, 0.62 mol), absolute alcohol (227 ml), toluene (115 ml) and 0.95 g conc. sulphuric acid were placed with a magnetic follower in a 1 litre round bottomed flask fitted with a short Vigreux column which was connected to a downward condenser and receiving flask. The mixture was heated with stirring until the malic acid had dissolved (oil bath 115°), then the oil bath temperature was lowered to 105°C whereupon a tertiary azeotrope distilled off at 75°. 300 ml azeotrope were collected over 96 g of anhydrous potassium carbonate and the distillation stopped. The distillate was thoroughly shaken with the potassium carbonate to remove water and the solid filtered off. The filtrate was replaced in the flask and distilled off again at 75°C (oil bath 105°). After all the azeotrope had distilled off the residue was allowed to cool then neutralised with 1.5 g of potassium carbonate. The solid was filtered off and the residue distilled to give (S)-diethyl malate as a colourless oil, (102.2 g, 87%) b.p. 120°, 0.1 mmHg; lit.[203] 145-148°, 1.4 mmHg $[\alpha]_D^{25}$ = -12.85° (C = 6.3, acetone), lit.[203] 15.9° (C = 6.3, acetone).

I.r. (film): 3500 (br.m), 2990 (m), 2940 (w), 2920 (w), 1740 (s), 1470 (w), 1450 (w), 1375 (m), 1350 (w), 1270 (s), 1220 (s), 1180 (s), 1105 (s), 1030 (s), 860 (s).

N.m.r. ($CDCl_3$, TMS): 1.28 (q, 6 H, 2 x $OCH_2\underline{CH}_3$), 2.80 (t, 2 H, $CH_2\underline{CH}_2CHOH$), 3.39 (br.s, 1 H, OH), 4.16 (q, 2 H, $O\underline{CH}_2CH_3$), 4.27 (q, 2 H, $O\underline{CH}_2CH_3$), 4.49 (t, 1 H, $\underline{H}COH$).

2-Methoxypropene

This was prepared according to the method of Newman and Vander Zwan[204] from 2,2-dimethoxypropane, in 75% yield, b.p. 36.5° 760 mmHg, lit. b.p. 37°[204].

N.m.r. (CCl_4, TMS): 1.76 (s, 3 H, $\underline{CH_3}C$), 3.48 (s, 3 H, $\underline{CH_3}O$-), 3.74 (s, 2 H, =$\underline{CH_2}$).

Lit. n.m.r. (CCL_4, TMS): 1.75 (s, 3 H, $\underline{CH_3}C$), 3.48 (s, 3 H, $\underline{CH_3}O$), 3.80 (s, 2 H, =$\underline{CH_2}$).

2-Methoxyprop-2-yl ether of diethyl malate(I)

To a 25 ml r.b. flask containing a magnetic stirrer was added (S)-diethyl malate (0.5 g, 6 mmol), 2-methoxypropene (7.5 ml) and a small drop of phosphorus oxychloride. A black oil formed immediately. The mixture was stirred for two hours then two drops of triethylamine were added. Evaporation of the oil afforded a viscous black residue from which only unreacted (S)-diethyl malate could be isolated.

2-Methoxyprop-2-yl ether of diethyl malate(II)

To a 25 ml r.b. flask containing a magnetic stirrer was added (S)-diethyl malic acid (0.5 g, 6 mmol), a solution of 2-methoxypropene (7 ml) in chloroform (7 ml), and a small drop of phosphor oxychloride. The solution was stirred for 2 h during which time the solution turned slightly brown in colour. The solution was stirred for a further 15 h, then the solution was evaporated to yield a thick black oil containing unreacted (S)-diethyl malate.

S-(-)-Butane-1,2,4-triol

This was prepared according to the procedure of Nakanishi *et al.*[209] in 58.9% yield, b.p. 140°, 0.1 mmHg, lit. b.p.[209] 145-148°, 1.4 mmHg.

I.r. (film): 3340 (brs), 2940 (brs), 2880 (brs), 1420 (brm), 1110 (brw), 1060 (brm), 980 (w), 945 (w), 905 (w).

N.m.r. (D_2O, TSS): 1.71 (m, 2 H, $HOCH\underline{CH_2}CH_2OH$), 3.58 (dq, 2 H, $\underline{CH_2}CH_2OH$), 3.76 (t, 2 H, $CH_2\underline{CH_2}OH$) 3.86 (m, 1 H, $-\underline{CH}OH$).

Lit. n.m.r.[209] (pyridine): 2.14 (m, 2 H), 3.97 (dd, 2 H), 4.17 (dt, 2 H), 5.38 (m, 1 H), 6.00 (s, 3 H). $[\alpha]_D^{25} = -17.33$ (C = 3, MeOH)

S-(-)-4-(2'-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolane

Butane-1,2,4-triol (28.9 g, 0.27 mol) was dissolved in acetone (200 ml). 0.5 ml of conc. sulphuric acid was added, and the mixture left stirring overnight. Anhydrous potassium carbonate (3 g) was added and the mixture stirred for 10 min. (to neutralise acid and dry the solution). Evaporation of the solvent afforded an orange oil which was distilled to give a colourless oil (31.0 g, 82.7%), b.p. 80°, 10 mmHg; lit. b.p. 87°, 22 mmHg[203], $[\alpha]_D^{25} = -1.29°$ (C = 4.6, methanol).

I.r. (film): 3430 (br.s), 2990 (s), 2940 (s), 2980 (s), 1455 (w), 1380 (s), 1370 (s), 1250 (s), 1220 (s), 1160 (s), 1060 (s), 990 (w), 960 (w), 940 (w), 860 (m), 820 (w), 795 (w).

N.m.r. ($CDCL_3$, TMS): 1.38 (s, 3 H, $-CH_3$), 1.44 (s, 3 H, $-CH_3$), 1.83 (dt, 2 H, J = 5 Hz & 26 Hz, $HOCH_2\underline{CH_2}-$), 2.63 (br.s, 1 H, $-\underline{OH}$), 3.61 and 4.10 (t, 1 H, ring-CH_2), 3.81 (t, 2 H, J = 6 Hz, $\underline{CH_2}OH$), 4.39 (p, 1 H, -OCH).

S-(+)-4-Benzyloxybutane-1,2-diol

S-(-)-4-(2'-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane (29 g, 0.199 mol) was stirred at 100°, (oil bath), with 50% aqueous sodium hydroxide (72 ml), benzyl chloride (50.6 g, 0.4 mol) and benzyl n-butyl ammonium bromide (2 g) for 14 h. After allowing to cool to r.t., water (72 ml) was added and the organic layer was separated. The remaining aqueous layer was extracted with ether (3 x 100 ml) and all organic fractions

combined and washed with water (3 x 200 ml). The solvent was removed from the ethereal solution to give a yellow oil of crude 4-(2'-benzyloxyethyl)-2,2-dimethyl-1,3-dioxolane. The crude material was stirred vigorously at 100° with 2 M sulphuric acid for 3 h, then allowed to cool to r.t. Water (72 ml) was added and the solution extracted with 40-60° petrololeum ether (3 x 100 ml) to remove dibenzyl ether and some benzyl alcohol. The aqueous phase was made alkaline with 50% aqueous sodium hydroxide (50 ml), then saturated with sodium chloride and extracted with ethyl acetate (3 x 100 ml). The combined organic fractions were dried ($MgSO_4$) and the solvent evaporated to give a yellow oil which was distilled to yield a colourless oil (28.4 g, 87%) b.p. 144-146°, 0.05 mmHg; $[\alpha]_D^{25}$ = +2.4° (C = 5, chloroform).

I.r.: 3400 (br.s), 3095 (w), 3060 (w), 3030 (w), 2930 (m), 2870 (m), 1500 (w), 1460 (m), 1365 (m), 1210 (w), 1100 (br,s), 910 (w), 870 (w), 740 (s), 700 (s).

N.m.r. ($CDCl_3$, TMS): 1.73 (m, 2 H, $CH_2\underline{CH_2}C$-OH), 3.55 (m, 4 H, $BzO\underline{CH_2}$ and $\underline{CH_2}OH$), 3.87 (m, 1 H, $\underline{H}C(OH)CH_2$), 4.50 (s, 2 H, $PhC\underline{H_2}O$), 7.30 (s, 5 H-$\underline{Ph}CH_2O$).

(S)-(-)-4-(2'-Benzyloxyethyl)-2-phenyl-1,3-dioxolane

4-Benzyloxybutane-1,2-diol (28 g, 0.14 mol) and benzaldehyde (14.84 g, 0.14 mol) were dissolved in benzene (600 ml) and 0.3 g of p-toluenesulphonic acid were added. The mixture was refluxed overnight with azeotropic removal of water (Dean & Stark apparatus), then allowed to cool to r.t. Potassium carbonate (3 g) was added and after 10 min. stirring was filtered off. The solvent was removed to yield an orange oil (38.5 g, 100%). The product was pure by t.l.c. [CH_2Cl_2, I_2, R_F 0.41] and its ^{1}H n.m.r. spectrum and therefore used without

further purification.

N.m.r. ($CDCl_3$, TMS): 1.99 (m, 2 H, $-CH_2\underline{CH_2}CHOH$), 3.62 (t, 2 H, $BzO\underline{CH_2}CH_2-$), 3.74 and 4.10 (t, 1 H, ring CH_2), 3.98 (s, 2 H, $PhC\underline{H_2}O$), 5.77 and 5.88 (s, both peaks together = 1 H, $PhC-\underline{H}$, 1 peak for each isomer), 7.31 and 7.44 (m, 8 H and 2 H respectively, ^1H of aromatic rings), $[\alpha]_D^{25} = -4.6$ (C = 5, $CHCl_3$).

S-(-)-2-Benzoyloxy-4-benzyloxy-1-bromobutane

N-Bromosuccinimide (24.92 g, 0.14 mol) was added in small amounts to a solution, cooled to 0°, of (S)-(-)-4-(2'-benzyloxyethyl)-2-phenyl-1,3-dioxolane (38 g, 0.14 mol) in carbon tetrachloride (300 ml). The reaction mixture was stirred in darkness at r.t. overnight to produce a yellow solid and orange solution. The solid was filtered off and the solution washed with an equal volume of saturated sodium bicarbonate to give a colourless solution which was dried ($MgSO_4$). Evaporation of the solvent yielded a pale yellow oil (46.86 g, 100%) pure by t.l.c. [CH_2Cl_2, I_2, R_F 0.44] and its ^1H n.m.r. spectrum, $[\alpha]_D^{25} = -13.6°$ (C = 5, chloroform).

I.r. (film): 3090 (m), 3060 (m), 3030 (m), 2960 (m), 2930 (m), 2860 (s), 1740 (v.s), 1610 (s), 1590 (m), 1570 (m), 1450 (v.s), 1360 (s), 1330 (s), 1275 (v.s), 1175 (s), 1085 (v.s), 1030 (s), 910 (m), 850 (m), 805 (m), 735 (s), 700 (s).

N.m.r. (CCl_4, TMS): 2.09 (q, 2 H, $OCH_2\underline{CH_2}CHO$), 7.60 (m, 4 H, CH_2Br and $BzO\underline{CH_2}$), 6.43 (s, 2 H, $PhC\underline{H_2}O$), 5.34 (p, 1 H, $CH_2\underline{CH}CH_2Br$), 7.19 (m, $\underline{Ph}CH_2O$), 7.42 (m, 3 H, $\underline{Ph}CO_2$), 7.97 (d, 2 H, $PhCO_2$).

S-(-)-(2 -Benzyloxyethyl)oxirane

S-(-)-2-Benzoyloxy-4-benzyloxy-1-bromobutane (40 g, 0.11 mol) was dissolved in ethane diol (80 ml). Powdered sodium hydroxide (8.8 g,

0.22 mol) was added and the mixture stirred at r.t. The reaction was followed by extracting aliquots of the reaction mixture with 30-40° petroleum ether, evaporation of the solvent and examination of the n.m.r. spectrum. After 17 h of stirring at r.t., the reaction was complete and the reaction mixture extracted with 30-40° petroleum ether (3 x 100 ml). The ethereal fractions were combined and evaporated to give a yellow oil which was purified by distillation to obtain a colourless oil (11.80 g, 70%), b.p. 120°, 10 mmHg $[\alpha]_D^{25} = -12.2^\circ$ (C = 5, chloroform).

N.m.r. (CCl_4, TMS): 1.74 (m, 2 H, $OCH_2\underline{CH_2}CH$), 2.37 (m, 1 H, 1'H of ring methylene group), 2.63 (t, 1 H, 1'H of ring methylene group), 2.92 (m, 1 H, methine H of ring), 3.53 (t, 2 H, $BzO\underline{CH_2}CH_2$), 4.47 (s, 2 H, $Ph\underline{CH_2}O$), 7.24 (s, 5 H, Ph, CH_2O).

Reaction of (S)-(-)-4-benzyloxybutane-1,2-diol with HBA

Hydrogen bromide-acetic acid (0.28 g, 0.75 mmol) was added to (S)-(-)-4-benzyloxybutane-1,2-diol (0.05 g, 0.25 mmol) with stirring. After stirring for 15 min. at r.t., water (5 ml), was added and the mixture neutralised with solid sodium carbonate. The neutral solution was extracted with ether (3 x 5 ml) and the extracts combined, dried and evaporated to give a colourless oil (96 mg, 96%). The ^1H n.m.r. spectrum showed the product to consist of benzyl bromide and (S)-2,4-diacetoxy-1-bromobutane (by comparison with reference spectra of the two compounds). No other compounds were present.

N.m.r. ($CDCl_3$, TMS) (S)-2,4-diacetoxy-1-bromobutane signals: 2.05 (s, 3 H, $BrCH_2CHO\underline{Ac}$-), 2.11 (s, 3 H, $\underline{Ac}OCH_2CH_2$-), 3.52 (m, 2 H, $-CH_2Br$), 4.13 (t, 2 H, CH_2OAc), 5.10 (p, 1 H, $BrCH_2\underline{CH}OAc$).

Benzylbromide signals: 4.50 (s, 2 H, $Ph\underline{CH_2}Br$) 3.32 (m, 5 H, Ph, CH_2Br).

TABLE 5.1

Effect on the ^{1}H n.m.r. Spectrum of RS-(2'-benzyloxyethyl)oxirane upon Addition of Europium(III) *Tris*[3-(heptafluorobutryl-d-camphorate)]

Amount shift reagent	0 mg	5 mg	10 mg
	t @ 2.63	m @ 2.98	2t @ 3.47 & 3.57
	d @ 4.47	brs @ 5.66	2d @ 6.78 & 6.85

Optically active shift reagent experiments on (2-benzyloxyethyl)oxirane

RS-(2 -Benzyloxyethyl)oxirane was prepared in exactly the same way as (S)-(2 -benzyloxyethyl)oxirane, but using racemic malic acid as starting material. RS-(2 -Benzyloxyethyl)oxirane (30 mg) was dissolved in deuterochloroform (0.5 ml) and the solution was transferred to a ^{1}H n.m.r. tube. After the n.m.r. spectrum has been taken, europium(III) *tris*-[3-(heptafluorobutryl)-d-camphorate] was added in successive 5 mg amounts, the ^{1}H n.m.r. spectrum being taken after each addition. The resulting peak splittings are given in Table 5.1.

(S)-(2 -Benzyloxyethyl)oxirane (30 mg) was dissolved in deuterochloroform (0.5 ml). The solution was treated in exactly the same way as for the racemic material. With scale expansion of the n.m.r. spectra at high sensitivity, no peaks due to the presence of (R)-(2 -benzyloxyethyl)oxirane could be detected, indicating the product to be optically pure within the limitations of the method, i.e. $\geq$ 98%.

Anhydrous copper(II) chloride

This was prepared from commercial material which was dried by refluxing in thionyl chloride. After 30 min. excess thionyl chloride was distilled off, final traces being removed *in vacuo*.

Dilithium tetrachlorocuprate

Anhydrous copper(II) chloride (0.16 g, 1.2 mmol) was quickly weighed and added to 12 ml of dry THF in a vial fitted with a suba seal. Oven dried lithium chloride (0.098 g, 2.4 mmol) was quickly added and the mixture stirred for 30 min. until both solids had dissolved. The resulting brown-red solution was kept under nitrogen and used immediately.

2-(7'-Benzyloxy-5'-hydroxyheptyl)-2-methyl-1,3-dioxolane

Dry magnesium turnings (1.5 g, 0.062 mol) were placed in a dry 3-necked r.b. flask fitted with nitrogen inlet, double surface condenser and self equilibriating dropping funnel. Dry THF (20 ml) was added to cover the magnesium and dibromoethane (0.5 ml) added to initiate the reaction. Upon heating with warm water (65°) the reaction commenced and 2-(3'-chloropropyl)-2-methyl-1,3-dioxolane (8.51 g, 0.052 mol) dissolved in 10 ml dry ether was added over a period of 5 min. to maintain refluxing. Upon completion of the addition, the reaction mixture was refluxed for 2 h to ensure complete formation of the Grignard reagent. The flask was cooled to -78° (acetone/dry ice bath) and freshly prepared 0.1 M solution of di-lithium tetrachlorocuprate in THF (2 ml) was added and left for a 1 h induction period[214]. S-(2-benzyloxyethyl)oxirane (9.4 g, 0.051 mol) was quickly added and the mixture was stirred whilst the cooling bath was allowed to warm to r.t. overnight. 10% (w/v) Aqueous ammonium chloride (25 ml) was added and the mixture extracted with ether (3 x 25 ml). The extracts were dried (anhydrous magnesium sulphate) and evaporated to give a pale green oil (15.2 g, 83.5%). T.l.c. [ether, I_2, R_F 0.36, product, 0.57 impurity] The oil was purified by flash column chromatography. An 80 cm column containing an 85 cm depth of Kiesgel 60, 230-400 mesh, was loaded with the product and eluted with ether at a rate of 2" per min. Fractions were collected (60 x 40 ml) and examined by t.l.c.

Fractions 21 onwards contained pure product and were combined and evaporated to give a colourless oil (10.5 g, 62% from S-(-)-(2-benzyloxyethyl)oxirane, pure by t.l.c. [ether, I_2, R_F 0.37], $[\alpha]_D^{25}$ = +6.4°, (C = 5, $CDCl_3$).

N.m.r. (CCl_4, TMS): 1.24 (s, 3 H, $-CH_3$), 1.26-1.69 (m, 10 H, $CH_2CHOH(CH_2)_4$), 2.43 (br.s, 1 H, OH), 3.63 (m, 3 H, HCOH and $BzOCH_2$),

3.85 (s, 4 H, OCH_2CH_2O), 6.49 (s, 2 H, $PhCH_2O$), 7.27 (s, 5 H, $\underline{Ph}CH_2O$). I.r. (film): 3450 (br.m), 3090 (w), 3075 (w), 3030 (w), 2990 (m), 2940 (s), 2870 (s), 1500 (w), 1455 (m), 1375 (m), 1315 (w), 1265 (w), 1255 (w), 1220 (m), 1090 (s), 1065 (s), 950 (w), 850 (w), 740 (m), 700 (m).

Fractions 1-21 were combined and evaporated to yield a colourless oil of unidentified material. Pure by t.l.c. [ether, I_2, R_F 0.53]

7,9-Dibenzyloxy-2-oxononane

2-(7'-Benzyloxy-5'-hydroxyheptyl)-2-methyl-1,3-dioxolane (0.886 g, 2.88 mmol) was stirred at 100° (oil bath) with 50% aqueous sodium hydroxide (5 ml), benzyl chloride (0.73 g, 5.76 mmol) and benzyl tri-n-butyl ammonium bromide (0.1 g) and the reaction followed by t.l.c. After 20 h no further change was observed although starting material was still present and the reaction mixture was worked up. Water (10 ml) was added to the reaction mixture which was then extracted with ether (3 x 20 ml) and the extracts combined, washed with water (3 x 60 ml), dried (anhydrous magnesium sulphate) and the solvent removed to yield a yellow oil, t.l.c. [ether, I_2, R_F 0.65 (dibenzyl ether), 0.56 (dibenzyloxy product), 0.37 (starting material).

The crude product was stirred vigorously at 100° with 2 M aqueous sulphuric acid (5 ml) for 2 h. Water (5 ml) was added and the mixture extracted with ether (3 x 20 ml). The organic fractions were combined, dried (anhydrous magnesium sulphate), and evaporated to give a yellow oil, t.l.c. [ether, I_2, R_F 0.54 (7,9-dibenzyloxy-2-oxynonane), 0.27 (9-benzyloxy-7-hydroxy-2-oxynonane), 0.65 (dibenzyl ether). The product was purified by silica gel chromatography 25 g silica gel were eluted with ether and 20 x 5 ml fractions collected. Fractions 1-9 were combined and evaporated to give 75.0 mg of dibenzyl ether, and a small amount of product t.l.c. [ether, I_2, R_F 0.65, 0.55).

N.m.r. ($CDCl_3$, TMS): 4.56 (s, 4 H, 2 x OCH_2Ph), 7.35 (m, 10 H, 2 x OCH_2Ph), plus trace of dibenzyloxy product.

Fractions 10-12 were combined and evaporated to yield 7,9-dibenzyl-2-oxononane containing 30% dibenzyl ether (0.32 g, 27.9%), t.l.c. [ether, I_2, R_F 0.65, 0.55].

N.m.r. ($CDCl_3$, TMS): 1.36 (m, 4 H, $CH_2CH_2CH_2COMe$), 1.54 (m, 2 H, $BzOCH_2CH_2CHOBz$), 2.11 (s, 3 H, $-CH_3$), 2.39 (t, 2 H, CH_2COCH_3), 3.58 (m, 3 H, $BzOCH_2$ and $OBzCH$), 4.59 (s, 2 H, $HCOCH_2Ph$), 4.70 (s, 2 H, CH_2OCH_2Ph), 7.34 (m, 10 H, 2 x OCH_2Ph) dibenzyl ether peaks occur at δ 4.54 and 7.36.

Fractions 13-20, combined and evaporated, yield 0.368 g (32%) of pure 9-benzyloxy-7-hydroxynonan-2-one. T.l.c. [ether, I_2, 0.27].

N.m.r. ($CDCl_3$, TMS): 1.45 and 1.59 (m, 6 H, $HOCHCH_2CH_2CH_2COMe$), 1.74 (q, 2 H, $BzOCH_2CH_2CHOH$), 2.13 (s, 3 H, CH_3), 2.43 (t, 2 H, CH_2COMe), 2.84 (br.s, 1 H, OH), 7.73 (m, 3 H, $BzOCH_2CHOH$), 6.54 (s, 2 H, $PhCH_2O$), 7.34 (s, 5 H, $PhCH_2O$).

4-Hydroxymethyl-2-phenyl-1,3-dioxane

(S)-(-)-Butane-1,2,4-triol (1 g, 9.4 mmol) was dissolved in benzene (20 ml), containing p-toluenesulphonic acid (0.015 g). Benzaldehyde (0.89 g, 9.5 mmol) was added and the mixture was refluxed overnight with azeotropic removal of water (Dean and Stark apparatus). After cooling to r.t., anhydrous potassium carbonate (0.5 g) was added and the mixture stirred for 10 min. Removal of the solid by filtration and evaporation of benzene afforded a yellow oil which was purified by distillation in a Kugelröhr apparatus (oven temp. 110°, 0.001 mmHg, (1.72 g, 93.9%).

N.m.r. ($CDCl_3$, TMS): 1.40 (d, 1 H, J = 11 Hz, axial H of $-O-CH_2CH_2CHCH_2OH$), 1.85 (dq, 1 H, J = 4 Hz, eq. H of $-O-CH_2CH_2CHCH_2OH$),

2.90 (br.s, 1 H, OH), 3.11 (d, 2 H, $C\underline{H}_2OH$), 3.93 (m, 2 H, $-O-C\underline{H}_2CHCH_2OH$), 4.25 (m, 1 H, $CCCH_2OH$), 5.50 (s, 1 H, $\underline{H}CPh$), 7.4 (m, 5 H, Ph). Peaks at 5.77 and 5.90 both singlets showed the presence of the *cis/trans*-isomer of 4-(2-hydroxyethyl)-2-phenyl-1,3-dioxolane present in ∿ 6% each.

4-Hydroxymethyl-2-phenyl-1,3-dioxane p-toluenesulphonate

Dry pyridine (5 ml) was placed in a 25 ml B-10, 3-necked flask fitted with dropping funnel, drying tube, stopper and magnetic follower. 4-Hydroxymethyl-2-phenyl-1,3-dioxane (1.72 g, 8.86 mmol) was dissolved in the pyridine and the solution cooled to -15° (ice/salt). A solution of p-toluenesulphonyl chloride (1.88 g, 8.86 mmol, recrystallised from 30-40° petroleum ether) in 5 ml dry pyridine was added dropwise, with stirring over 10 min. The cooling bath was removed and the mixture stirred at r.t. until precipitation of pyridinium hydrochloride ceased (2 h). Water (50 ml) was added and the mixture extracted with ether (50 ml). The ethereal layer was washed with 2 M hydrochloric acid (3 x 50 ml), saturated sodium carbonate (1 x 50 ml) and brine (50 ml), and dried (anhydrous magnesium sulphate) to give, upon evaporation of solvent, a pale yellow oil which slowly crystallised at 0° (1 week). Recrystallisation from 30-40° petroleum ether produced white crystals (2.42 g, 78.5%) pure by t.l.c. [dichloromethane, I_2, R_F = 0.288]. N.m.r. ($CDCl_3$, TMS): 1.52 (d, 1 H, J = 10, axial ^{1}H of $-O-CH_2C\underline{H}_2CHCH_2OH$), 1.75 (m, 1 H, J = 4, equatorial ^{1}H of $-O-CH_2C\underline{H}_2CHCH_2OH$), 3.87-4.29 (m, 5 H, $\underline{H}CCH_2OTs$, $-C\underline{H}_2OTs$ and $OC\underline{H}_2CH_2CHCH_2OH$), 5.43 (s, 1 H, $\underline{H}CPh$), 7.24 (d, 2 H) and 7.76 (d, 2 H)- AB quartet of aromatic protons of Ts group, 7.32 (m, 5 H, $HC\underline{Ph}$).

(S)-2,4-Diacetoxy-1-bromobutane

Hydrogen bromide-acetic acid (32.1 g, 0.135 mol) was added to (S)-(-)-butane-1,2,4-triol (4.8 g, 0.045 mol) with stirring. After stirring for 30 min. at room temperature, water (40 ml) was added and the mixture neutralised with solid sodium carbonate. The neutral solution was extracted with ether (3 x 50 ml), and the extracts, combined dried and evaporated to give an orange oil. The oil was distilled to give a colourless oil (8.99 g, 80%).

N.m.r. ($CDCl_3$, TMS): 2.05 (s, 3 H, $-CH_2OAc$), 2.10 (s, 3 H, $HCOAc$), 3.53 (m, 2 H, $-CH_2Br$), 4.13 (t, 2 H, CH_2OAc), 5.10 (m, 1 H, $HCOAc$).

CHAPTER 5 - REFERENCES

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CHAPTER 6

THE SYNTHESIS OF (S)-LIPOIC ACID FROM (S)-METHIONINE

6.1 INTRODUCTION

An outline of the proposed route for the synthesis of (S)-lipoic acid from (S)-methionine is given in Chapter 2 (Scheme 2.5).

6.2 SYNTHESIS OF (S)-2-HYDROXY-4-METHYLTHIOBUTANOIC ACID

The proposed route to (S)-lipoic acid involves the synthesis of (S)-2-hydroxy-4-methylthiobutanoic acid from (S)-methionine. The preparation of this compound in 15% yield by reaction of the diazotate of (S)-methionine with hydroxide ions has been reported by Steadman *et al.*[216]. If this reaction is to be useful in the synthesis of (S)-lipoic acid it must occur with complete retention of configuration. Although Steadman did not check the optical purity of the α-hydroxyacid he obtained, there is reason to suppose that it will be formed with complete retention of configuration. In 1936, Akobe[217] showed that the 2-hydroxy-4-methylthiobutanoic acid, formed by reaction of the diazotate of (S)-methionine with hydroxide ions had the same optical rotation as 2-hydroxy-4-methylthiobutanoic acid produced by an enzymic degradation of methionine with *bactilis subtilis* and presumed to have the L-configuration. Work by Brewster *et al.*[218] showed conclusively that complete retention of configuration occurs during the deamination of α-amino acids.

Steadman's procedure[216] for deaminating (S)-methionine was followed and gave (S)-2-hydroxy-4-methylthiobutanoic acid in *ca.* 15% yield. This product is isolated by extraction into ether from an aqueous reaction mixture. The use of dichloromethane instead of ether gave an oil containing at least six products by t.l.c. Several other attempts to improve the efficiency of extracting (S)-2-hydroxy-4-

methylthiobutanoic acid from the reaction were made but no significant improvement over the original procedure was achieved. The poor yield from the diazotisation could be tolerated because this step is at the beginning of the synthesis and (S)-methionine is extremely cheap and available in kilogram quantities[219]. 2-Hydroxy-4-methylthiobutanoic acid could be made conveniently in 15 g quantities starting from 100 g methionine.

6.3 REDUCTION OF 2-HYDROXY-4-METHYLTHIOBUTANOICACID

Steadman made (S)-4-methylthiobutane-1,2-diol in good yield by direct reduction of (S)-2-hydroxy-4-methylthiobutanoic acid using a three times excess of lithium aluminium hydride. He also achieved a good yield of the diol by reduction of the methyl ester of the hydroxy acid with 1 mole equivalent of reducing agent. Initially we used reduction of the methyl ester with lithium aluminium hydride and obtained 70% of pure diol. The drawback to this approach is that the methyl ester, prepared by reaction of the hydroxy acid with methanolic hydrogen chloride, was obtained in a yield of only 38%. Subsequently, reduction of the sodium salt of (S)-2-hydroxy-4-methylthiobutanoic acid, easily prepared in 48% yield by the general procedure of Childers and Struthers[220], was tried. The salt was reduced with slightly more than one equivalent of lithium aluminium hydride in THF, and gave pure diol in excellent yield (92%).

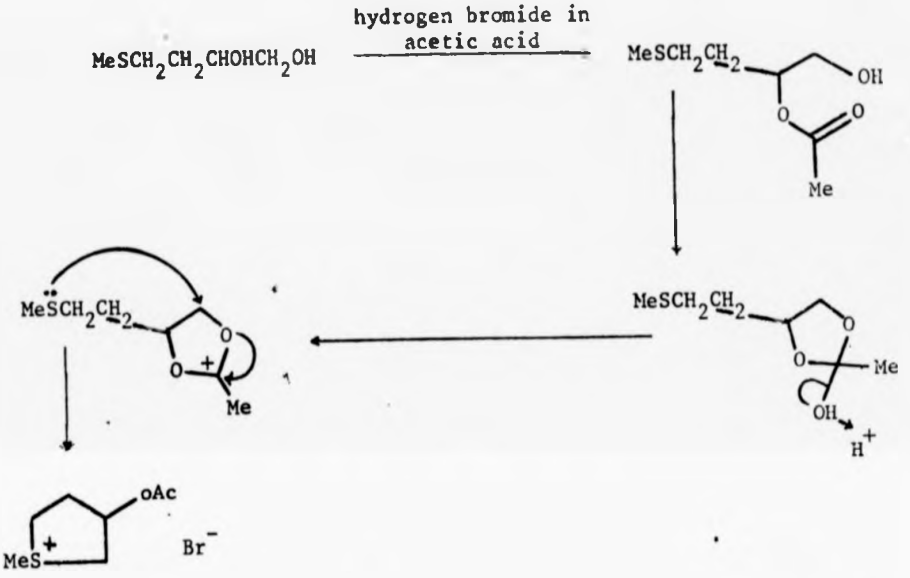
6.4 ATTEMPTED PREPARATION OF (S)-2'-METHYLTHIOETHYLOXIRANE

The preparation of (S)-2'-methylthioethyloxirane from (S)-4-methylthiobutane-1,2-diol was expected to be straightforward using hydrogen bromide in acetic acid to generate an acetoxybromide which would be cyclised to the oxirane by treatment with base. However, when the

reaction of (S)-4-methylthiobutane-1,2-diol and hydrogen bromide-acetic acid as solvent was carried out in an n.m.r. tube, ^1H n.m.r. spectral data indicated the almost instantaneous formation of an unexpected product, (S)-S-methyl-3-acetoxytetrahydrothiophenium bromide. This compound must have arisen by the intramolecular capture of an intermediate 1,3-dioxolan-2-ylium ion by the lone pair electrons of the sulphur atom, in preference to an intermolecular capture by the bromide ion present in the reaction mixture (Scheme 6.1). The reaction was repeated on a larger scale and the usual procedure modified to allow hydrolysis of (S)-S-methyl-3-acetoxytetrahydrothiophenium bromide to (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide. This was isolated as extremely pure needle shaped crystals. The ^1H n.m.r. spectrum of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide showed singlet peaks at δ 3.08 and 2.93, which together integrated for three protons. This indicated the presence of two diastereoisomers due to the different configurations adopted by the lone pair electrons and methyl group at the chiral sulphur atom (Scheme 6.2). These isomers were present in a 1:3 ratio, but during purification, the major isomer was found to preferentially crystallise and two recrystallisations gave a sample of one pure isomer.

There was not time to examine in detail the reaction of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide with base, which, in principle, should yield the desired (S)-2'-methylthioethyloxirane (Scheme 6.3). When an equimolar amount of potassium hydroxide in methanol was added to (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide no precipitation of potassium bromide (indicating the formation of the oxirane derivative) occurred. After overnight reflux an oil was isolated from the reaction mixture. The ^1H n.m.r. spectrum of this product showed it to consist of mainly starting material but two singlets at

SCHEME 6.1

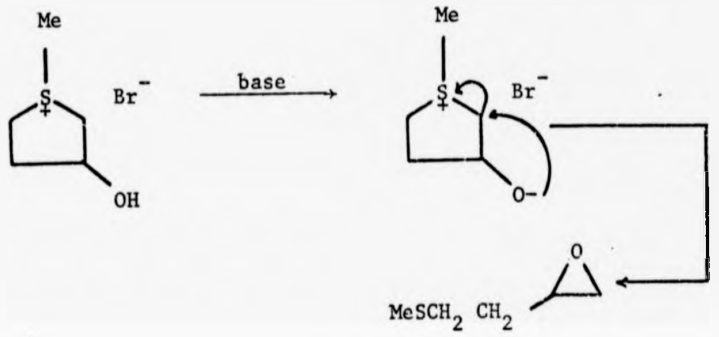


Mechanism of formation of (S)-S-methyl-3-acetoxytetrahydrothiophenium bromide from (S)-4-methylthiobutane-1,2-diol and hydrogen bromide – acetic acid

SCHEME 6.2

Configurations of the sulphur atom of (S)-S-methyl 3-hydroxytetrahydrothiophenium bromide

SCHEME 6.3



Possible reaction of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide with base

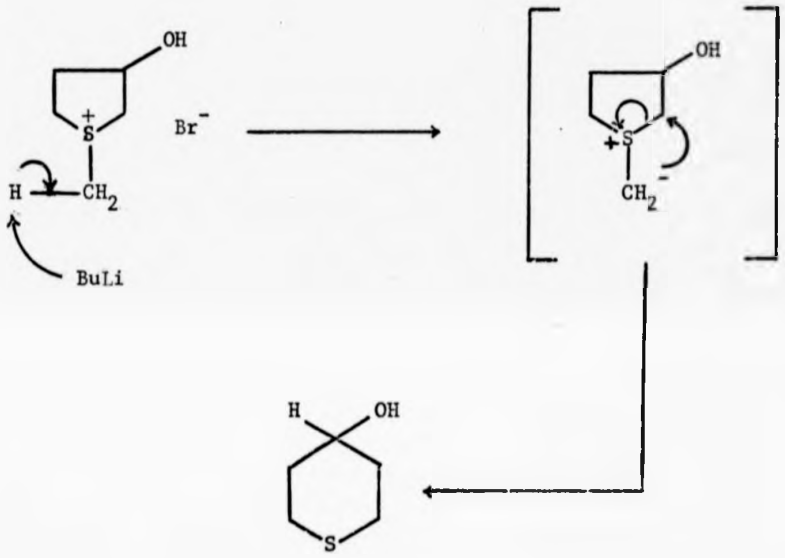
δ 3.22 and 2.1 could indicate the methoxy and methylthio groups, respectively, of (S)-2-hydroxy-1-methoxy-4-methylthiobutane. This may have arisen by the attack of methoxide on (S)-2'-methylthioethyl-oxirane that may have been formed. A further attempt to make (S)-2'-methylthioethyloxirane from (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide was made using a powerful base. An oil was obtained in good yield from the reaction of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide with an equimolar amount of butyllithium in hexane but its ^{1}H n.m.r. spectrum lacked a signal from a methylthio peak. This implied that the starting material had been demethylated and further study of the ^{1}H n.m.r. spectrum suggested that the product was (S)-4-hydroxytetrahydrothiopyran. Such a compound could arise *via* deprotonation of the methyl group of the starting material. The resulting intermediate could undergo a rearrangement to (S)-4-hydroxytetrahydrothiopyran (Scheme 6.4). Such reactions are known to occur: for example, the Stevens rearrangement of sulphonium and oxo-quaternary ammonium salts to thio and amino-ketones, under strongly basic conditions.

Although the failure of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide to form an oxirane under basic conditions was surprising, further investigation of this compound showed it to possess other unexpected chemical properties.

When crystals of the compound were heated under vacuum, an oil distilled which was characterised as pure (S)-3-hydroxytetrahydrothiophene. A likely mechanism for this reaction is that the methyl group of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide is attacked by bromide to give methyl bromide and (S)-3-hydroxytetrahydrothiophene.

^{1}H n.m.r. spectra of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide in deutero-DMSO, incubated at 60°, showed that after 2 hours the pure isomer used at the start had partially isomerised. This was

SCHEME 6.4



A Possible Mechanism for the Formation of
(S)-4-Hydroxytetrahydrothiopyran from the action
of Butyllithium on
(S)-S-Methyl-3-hydroxytetrahydrothiophenium Bromide

indicated by the appearance and growth of a singlet at δ 2.93 with the simultaneous decline of the original thiomethyl singlet at δ 3.14. After 8 hours the original singlet had disappeared and complete inversion of the configuration of the sulphur atom had occurred. After a further 7 hours, signals for (S)-3-hydroxytetrahydrothiophene were observed and after 36 hours the thiophenium bromide had been consumed. At this stage the ^{1}H n.m.r. spectrum consisted of peaks consistent with (S)-3-hydroxytetrahydrothiophene and there was a large singlet at δ 3.59 which confirmed that methyl bromide is produced (Table 6.1).

6.5 IMPROVEMENT AND LIMITATIONS OF THE SYNTHESIS

An improvement in the yield of (S)-2-hydroxy-4-methylthiobutanoic acid from (S)-methionine by the use of other methods of converting an amino group to a hydroxy function might also result in a complex mixture of products. The presence in methionine of the thiomethyl group, a readily oxidised function, makes it difficult to achieve selective conversion of amino to hydroxy. Inactivation of the thiomethyl group of methionine by its conversion to sulphone or sulphoxide could circumvent this problem. Reduction of, e.g. the sulphoxide, is a possibility, especially as there are many procedures for this type of reaction[221-225]. However, this approach would add two steps to the synthesis. Actually, the ^{1}H n.m.r. spectrum of the crude product from diazotisation of methionine showed a resonance indicative of a methylsulphoxide group.

Other methods of stereoselective oxirane synthesis from diols are likely to produce (S)-S-methyl 3-acetoxytetrahydrothiophenium bromide from (S)-4-methylthiobutane-1,2-diol. This is because activation of the primary hydroxyl, required as the basis for

TABLE 6.1

Effect on the ^1H n.m.r. Spectrum of (S)-S-methyl 3-hydroxytetrahydrothiophenium Bromide in Deuterated DMSO

Time/h	Change in n.m.r. Spectrum	Inference
1	Appearance of singlet at δ 2.93	Start of isomerisation
2	Singlets at δ 3.14 and 2.93 equal area	Equal amounts of the two isomers
6	Complete disappearance of singlet at δ 3.14. Singlet at 2.93 integrates for 3 H	Complete inversion of configuration
20	Small peak at δ 2.58	Presence of MeBr
	Peaks at 2.04 (m, 2 H), 2.84 (4 H, m), 4.54 (m, 1 H), 5.20 (brs, 1 H)	Formation of 3-hydroxy-tetrahydrothiophenium bromide and methyl bromide complete
	Singlet at δ 2.5	

oxirane formation, will encourage intramolecular attack by sulphur leading to the 5-membered thiophenium species. Treatment of (S)-S-methyl 3-hydroxytetrahydrothiophenium bromide with a hindered base such as lithium 2,2,6,6-tetramethylpiperidide may help to avoid deprotonation of the methylthio group and favour oxirane formation.

Methionine possesses three reactive functional groups which limits its use as a starting material. The amino and carboxyl groups can act as both electrophiles ($\overset{+}{N}H_3$, CO_2H) or nucleophiles (NH_2, CO_2^-) and their reactivity may in some circumstances be difficult to differentiate from that of the thiomethyl group. The nucleophilic properties of the thiomethyl group of methionine have been shown to become involved in unwanted cyclisation and other side reactions.

Methionine has proved to be a difficult starting material to work with and it must be concluded that without a lot more work on the preparation of 2'-methylthioethyloxirane from methionine both tartrate diesters and malic acid offer more viable starting materials for the synthesis of lipoic acid in enantiomerically pure form.

EXPERIMENTAL

(S)-2-Hydroxy-4-methylthiobutanoic acid

This was synthesised by the procedure of Steadman *et al.*[216] to give (S)-2-hydroxy-4-methylthiobutanoic acid, b.p. 150-152° at 8 mmHg.

N.m.r. (60 MHz, $CDCl_3$, TMS): 2.12 (s and m, 5 H, CH_3S- and $-CH_2CH_2CHOH$), 8.68 (t, 2 H, CH_3SCH_2-), 4.43 (dd, 1 H, $HC(OH)CO_2H$), 5.80 (brs, 2 H, -OH and $-CO_2H$).

Four runs were carried out starting with 100 g of (S)-methionine each. The average yield was 15%.

Methyl (S)-2-hydroxy-4-methylthiobutanoate

(S)-2-hydroxy-4-methylthiobutanoic acid (15.94 g, 0.1 mol) was dissolved in methanol (122 ml) and acetyl chloride (9.9 ml) was added so as to give a 3% solution of hydrogen chloride. The reaction was followed by t.l.c. (dichloromethane/15% methanol, iodine, R_F acid 0.13, ester 0.83). After 3 days at r.t. the reaction had finished and the mixture was neutralised by the addition of solid potassium carbonate. The solid material was filtered off and the solvent was removed from the filtrate to give a residue which was distilled, yielding pure methyl ester b.p. 70-74° at 0.05 mmHg, 38.2%.

N.m.r. (60 MHz, $CDCl_3$, TMS): 2.10 (s and m, 5 H, CH_3S- and $-CH_2CHOH$), 2.66 (t, 2 H, CH_3SCH_2-), 3.0 (brs, 1 H, -OH), 3.80 (s, 3 H, $-CO_2CH_3$), 4.38 (m, 1 H, HCOH).

(S)-4-Methylthiobutane-1,2-diol

This was prepared from methyl (S)-2-hydroxy-4-methylthiobutanoic acid by the method of Steadman *et al.*[216] in 72% yield, b.p. 150-152 at 8 mmHg, lit. b.p. 100-102.5°, 0.2 mmHg.

T.l.c. (dichloromethane/15% methanol, iodine, R_F 0.52).

N.m.r. (60 MHz, $CDCl_3$, TMS): 1.79 (t, 2 H, $-CH_2\underline{CH_2}CHOH$), 1.11 (s, 3 H, $\underline{CH_3}S-$), 2.62 (t, 2 H, $CH_3S\underline{CH_2}-$)), 3.58 (d, 1 H, $-CH_2\underline{CH}OH$), 4.50 (brs, 2 H, 2 x $-\underline{OH}$).

Sodium (S)-2-hydroxy-4-methylthiobutanoate

(S)-2-Hydroxy-4-methylthiobutanoicacid (15 g, 0.10 mol) was added to water (100 ml) in a 2 l beaker and the solution made neutral to universal indicator paper, with sodium hydroxide solution (25%, w/v). The water was slowly evaporated on a hot-plate until a brown gum was obtained. When the residue had cooled to r.t. acetone (600 ml) was slowly added with vigorous stirring. The residue did not crystallise but formed a thick sticky mass which was cooled to -78° (acetone/dry-ice) and then allowed to stand at r.t. As the mixture gradually warmed the sides of the beaker were scratched and very fine white crystals formed. When crystallisation was complete, the crystals were filtered off, washed with ether and dried in an oven at 70° to give the sodium salt (5.56 g, 48%).

N.m.r. (60 MHz, D_2O, DSS): 1.84-2.09 (s and m, 5 H, CH_3S- and $CH_2\underline{CH_2}CHOH$), 2.60 (t, 2 H, $CH_3S\underline{CH_2}-$), 4.12 (t, 1 H, $HO\underline{H}CCO_2$).

(S)-4-methylthiobutane-1,2-diol from sodium (S)-2-hydroxy-4-methylthiobutanoate

Sodium (S)-2-hydroxy-4-methylthiobutanoate (5.5 g, 0.032 mol) was carefully added, in small portions, to a suspension of lithium aluminium hydride (1.85 g, 0.048 mol) in dry THF (360 ml), under a nitrogen atmosphere. When the addition was completed, the mixture was refluxed overnight then allowed to cool to r.t. A mixture of water (1.85 ml) and THF (1.85 ml) was carefully added to the reaction flask with stirring followed by the addition of aqueous sodium hydroxide

(15%. 1.85 ml) and then more water (9 ml). The white granular precipitate was filtered off and washed with THF (4 x 50 ml). The combined filtrates were dried (anhydrous magnesium sulphate) and evaporated to give an oil which was distilled to give a product, identical to that obtained by reduction of methyl (S)-2-hydroxy-4-methylthiobutanoic acid, in 92% yield.

Reaction of (S)-4-methylthiobutane-1,2-diol with hydrogen bromide-acetic acid (n.m.r. scale)

Hydrogen bromide-acetic acid (48% w/v, 0.8 g) was placed in an n.m.r. tube to give a depth of liquid of 3 cm. (S)-4-Methylthiobutane-1,2-diol (73 mg, 0.5 mmol) was added to the tube and the n.m.r. spectrum was taken. The resulting spectrum showed that an almost instantaneous reaction had occurred and there was no further change after 24 h. The spectrum indicated the formation of (S)-3-acetoxy-1-methylthiophenium bromide, by peaks at δ 2.21 (s, 3 H, OAc), 2.70 (m, 2 H, $CH_3SCH_2\underline{CH}_2$-), 3.32 (s, 3 H, $\underline{CH}_3S^{\oplus}$), 3.91 (m, 4 H, $-CH_2\overset{\oplus}{S}CH_2$-), 6.0 (brm, 1 H, $\underline{H}COAc$).

(S)-S-Methyl-3-hydroxytetrahydrothiophenium bromide

(2S)-4-Methylthiobutane-1,2-diol (1.15 g, 8.45 mmol) was stirred with hydrogen bromide-acetic acid (5.99 g, 23.35 mmol) for 30 min. Water (20 ml) was then added and the mixture was neutralised with an aqueous solution of potassium hydroxide (1 m). The water was evaporated and the residue taken up in methanol (10 ml).

The insoluble white potassium bromide (from neutralisation of the hydrogen bromide-acetic acid) was filtered off and discarded. Methanol was evaporated from the filtrate to yield an oil which crystallised on standing overnight at r.t. The pale brown solid was recrystallised from the minimum amount of boiling ethanol to give white needle-shaped crystals of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide

(0.87 g, 52.0%) m.p. 157°, $[\alpha]_{25}^{D}$ = -20.78° (C = 4.09, water).

N.m.r. (60 MHz, D_2O, DSS): 2.40 (m, 2 H, $-CH_2\underline{CH_2}CHOH$), 3.08 (s, 3 H, $\underline{CH_3}\overset{+}{S}-$), 3.48-4.08 (m, 4 H, $-\underline{CH_2}CH_2CHOH$ and $CH_3\overset{+}{S}\underline{CH_2}CHOH$), 5.04 (m, 1 H, $-\underline{CH}OH$).

I.r. (mull): 3220 (brm), 2960 (s), 2930 (s), 2895 (s), 2860 (s), 1460 (m), 1420 (m), 1410 (w), 1380 (m), 1345 (w), 1315 (m), 1270 (w), 1250 (w), 1210 (w), 1140 (w), 1115 (w), 1060 (s), 1040 (s), 990 (s), 950 (w), 935 (w), 810 (w).

Found (CHN): C 30.33, H 5.54, Br 39.86, S 15.96%; $C_5H_{12}BrOS$ (MW 199) requires C, (30.15, H 5.57, Br, 40.13, S 16.10%).

Thermolysis of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide:

(S)-S-methyl-3-hydroxytetrahydrothiophenium bromide (0.5 g, 2.5 mmol) was placed in a Kugelröhr distillation apparatus which was evacuated to 10 mmHg. The solid material was heated at 140° whilst a colourless oil was distilled into a cooled receiving flask (0.25 g, 100%) b.p. 92°, 11.5 mm (lit. value 90° @ 12 mm[221]).

N.m.r. (60 MHz, CCl_4, TMS): 2.06 (m, 2 H, ring methylene), 2.84 (m, 4 H, 2 x $-S\underline{CH_2}$), 4.54 (m, 1 H, $-\underline{CH}$), 5.20 (brs, 1 H, $\underline{OH}$).

Lit. value[222] (neat) 4.48 (m, 1 H), 4.63 (brs, 1 H, $\underline{OH}$), 3.00 (m, 4 H), 2.08 (m, 2 H).

I.r. (film): 3380 (brs), 2940 (s), 1430 (s), 1336 (s), 1262 (s), 1190 (w), 1025 (s), 550 (s), 830 (w), 780 (s), 760 (s).

Lit. value[222] i.r. 3382 (OH), 2943, 1451, 1336, 1262, 1195, 1027, 956, 830.

Solvolysis of (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide in deuterated DMSO: (S)-S-methyl-3-hydroxytetrahydrothiophenium bromide (70 mg, 0.34 mmol) was weighed into an n.m.r. tube and deuterated DMSO (0.5 ml) was added to form a solution. The n.m.r. tube was incubated at 60° and a spectrum was taken every hour for 6 h and then after 20 h.

N.m.r. 10 min. (pure (S)-5-methyl-3-hydroxytetrahydrothiophenium bromide)

2.32 (m, 2 H, ring methylene), 3.14 (s, 3 H, $\underline{CH_3}S$-), 3.48 (m, 4 H,

2 x -$\underline{CH_2}S$ of ring), 4.83 (brm, 1 H, $\underline{CH}$ of ring), 5.35 (brs, 2 H,

2 x $\underline{OH}$).

Results shown in Table 6.1.

CHAPTER 6 - REFERENCES

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CHAPTER 7

AN APPROACH TO THE SYNTHESIS OF 8-METHYLLIPOIC ACID

7.1 INTRODUCTION

A possible synthesis of 8-methyllipoic acid from methyloxirane and phenyl lithium has been discussed in Chapter 2. Stereoselective reduction of the proposed intermediate 8-hydroxy-6-oxononanoic acid is crucial to this synthesis. A study of the reduction of 4-hydroxy-2-oxopentane under various conditions has been made as a model for this reduction.

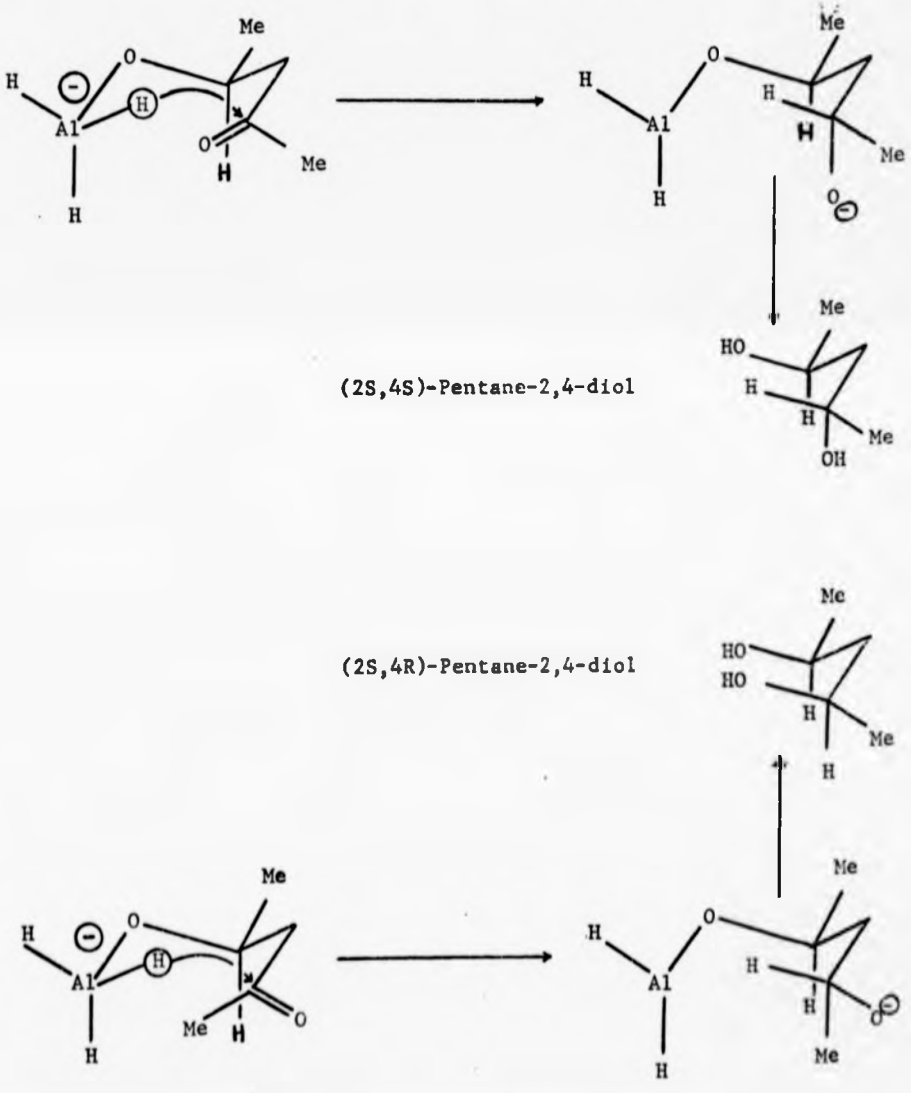
7.2 STEREOCHEMISTRY OF THE PROPOSED SYNTHESIS OF 8-METHYLLIPOIC ACID

8-Methyllipoic acid possesses two asymmetric centres and therefore can exist as four isomers (two diastereoisomeric pairs). The object of the work was to find a stereoselective reducing agent which would make possible the synthesis of both diastereoisomers having configuration at C-6 corresponding to that of natural lipoic acid. In the proposed synthesis, the configuration at C-8 of 8-methyllipoic acid, will be determined by the configuration of the starting methyloxirane used, whereas stereoselective reduction of optically active 8-hydroxy-6-oxononanoic acid will give control over the configuration at C-6 of 8-methyllipoic acid. If a reagent reduces the ketone group of (R)-8-hydroxy-6-oxononanoic acid with creation of predominantly an R-asymmetric centre and another reducing agent generates mainly an S-asymmetric centre then both the (6R,8R)- and the (6S,8R)-isomers of 6,8-dihydroxynonanoic acid would become available. Similarly, the (6S,8S)- and (6R,8S)-isomers of the dihydroxy acid would be available from (S)-8-hydroxy-6-oxononanoic acid.

7.3 POTENTIAL STEREOSELECTIVE REDUCING AGENTS AND THE THEORY OF THEIR ACTION

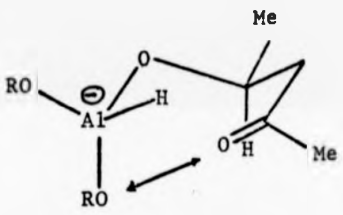
Considering the mechanism of reduction of β-hydroxyketones by reducing agents available to the synthetic organic chemist, metal aluminium hydrides or borohydrides seemed to offer the best opportunity for stereochemical control during the reduction. For example, in the reduction of a β-hydroxyketone by lithium aluminium hydride the first step should be coordination of the hydroxy group with evolution of hydrogen. A hydride ion is then transferred to the carbonyl carbon atom and a cyclic salt is formed by coordination of the resulting anion to the aluminium atom. Hydrolysis of this complex liberates the free diol. The hydride transfer is the crucial step of the reduction and is where stereochemical control may be effected, leading to either an R- or S-centre (Scheme 7.1). It may be possible to direct the formation of one particular isomer by using alkoxy derivatives of lithium aluminium hydride. The hydrogen atoms of lithium aluminium hydride can be easily replaced with alkoxy groups by reaction with an alcohol[226]. The reaction of one, two, three or four mole equivalents of alcohol with lithium aluminium hydride replaces one, two, three or four of the hydrogen atoms respectively. As two hydrogen atoms are required for the reduction of 4-hydroxy-2-oxopentane, two of the 4 hydrogen atoms of lithium aluminium hydride can be replaced by alkoxy groups. The relative stabilities of the rotamers shown in Scheme 7.2 should be governed by steric and polar effects within each species. These effects may be susceptible to changes of the cation and solvent. By examining a series of alkoxy aluminohydrides, under a variety of conditions, complete stereochemical control over the reduction of 8-hydroxy-6-oxononanoic acid may eventually be achieved.

SCHEME 7.1

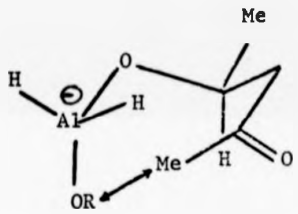


Proposed Mechanism of the Reduction of 4-hydroxy-2-oxopentane to (R,S)- and (S,S)-pentane-2,4-diol

SCHEME 7.2



Polar Repulsion



Steric Hindrance

Possible Polar and Steric Effects in

. Rotational Isomers Derived from

4-Hydroxy-2-oxopentane and $LiAlH_2(OR)_2$

7.4 A MODEL FOR THE REDUCTION OF 8-HYDROXY-6-OXONONANOIC ACID

Initial experiments on the proposed stereoselective reduction were performed using 4-hydroxy-2-oxopentane. There are a number of advantages in the use of this compound:

(a) The lack of functional groups other than the *sec*-hydroxyl and oxo functions ensures the absence of confusing side reactions and by-products which have nothing to do with the reduction of the carbonyl group.

(b) The ratio of *meso*- and *rac*-pentan-2,4-diol formed by the reduction will be a measure of the stereoselectivity of the reducing agent (see Section 7.5 for details).

(c) *Rac*-4-hydroxy-2-oxopentane has previously been synthesised in one step[227,228] and is therefore readily available, whereas 8-hydroxy-6-oxononanoic acid is available only by lengthy synthetic procedures (Chapter 1).

(d) Spectral data will be simpler allowing easier identification of stereoisomeric products.

(e) Pentan-2,4-diol has been synthesised and separated into its *meso*- and *rac*-components. Pure samples of *meso*- and *rac*-pentan-2,4-diol could therefore be made and characterised and used to establish a method of analysis for the reduction products of 4-hydroxy-2-oxopentane.

7.5 STEREOCHEMISTRY OF THE REDUCTION OF 4-HYDROXY-2-OXOPENTANE

If the R-isomer of *rac*-4-hydroxy-2-oxopentane is reduced with complete stereoselectivity (i.e. to the R,R-diol only), then the S-isomer of the *rac*-4-hydroxy-2-oxopentane, being of opposite configuration, will be reduced to the S,S-diol and a racemic mixture of R,R- and S,S-diol is obtained. However, if completely

stereoselective reduction of the opposite stereochemistry occurs, the R-isomer in *rac*-4-hydroxy-2-oxopentane would form R,S (*meso*)-diol and the S-isomer the S,R(*meso*)-diol and a product consisting of *meso*-diol only would result. Thus, the extent of the stereoselectivity of the reduction of *rac*-4-hydroxy-2-oxopentane is measurable by the ratio of *rac*- and *meso*-diols in the product.

7.6 SYNTHESIS OF *Rac*-4-HYDROXY-2-OXOPENTANE

4-Hydroxy-2-oxopentane was prepared by the base-catalysed condensation of acetaldehyde with acetone. When sodium phenoxide was used as the catalyst only a 17% yield of product was obtained which distilled over a 10° range and was shown by ^{1}H n.m.r. spectroscopy to consist of a mixture of approximately equal amounts of 4-hydroxy-2-oxopentane and 4-hydroxy-4-methyl-2-oxopentane. The latter must have been formed by the base-catalysed condensation of two molecules of acetone. Presumably, the low yield was due to the self-condensation of acetaldehyde, the resulting polymeric product not being isolated. Catalysis with sodium hydroxide gave a yield of 60% for crude product which consisted of equal amounts of 4-hydroxy-4-methyl-2-oxopentane and 4-hydroxy-2-oxopentane. These products could not be separated by distillation, even with the use of a fractionating column filled with glass helices. Neither of the reported methods[227,228] for the preparation of 4-hydroxy-2-oxopentane, on which this work was based, mentioned the fact that a large amount of by-product was formed. 4-Hydroxy-4-methyl-2-oxopentane and 4-hydroxy-2-oxopentane could not be sufficiently resolved by t.l.c. to suggest their separation by column chromatography and so a spinning band distillation was employed. The crude product was distilled under vacuum in a Nester and Faust spinning band distillation apparatus. The conditions used (see experimental) gave a 65% recovery

(by weight) of material of which 13% was pure 4-hydroxy-4-methyl-2-oxopentane (by ^{1}H n.m.r. spectroscopy) and 16% was > 90% pure 4-hydroxy-2-oxopentane. Although the overall yield for pure product was fairly low, enough material was obtained for use in the stereochemical reduction experiments.

An attempt was made to separate 4-hydroxy-4-methyl-2-oxopentane and 4-hydroxy-2-oxopentane by exploiting different rates of dehydration. The mixture was refluxed with benzene in a Dean and Stark apparatus with an anhydrous acid. Although 4-hydroxy-4-methyl-2-oxopentane should dehydrate fastest because it possesses a tertiary hydroxy group which should be readily eliminated, little dehydration occurred under the reaction conditions employed.

7.7 THE PREPARATION OF PURE SAMPLES OF *Meso*- AND *Rac*-PENTANE-2,4-DIOL

Pentane-2,4-diol has been studied as a model system for spectroscopic and chemical studies[229-232] relating to polymers. There are therefore a number of methods in the literature for its synthesis, but most of them are unsatisfactory. For example, reaction of 3-hydroxybutanal with methyl magnesium iodide[233] afforded the diol in a yield of 20%. Reduction of the readily available acetylacetone with sodium in alcohol also gave a low yield[234]. Nickel-hydrogen reduction of acetylacetone gave a 75% yield of diol but the reaction was inconvenient to perform because hydrogen pressures of 1000-2000 psi are required for 5 hours[234]. It was decided to use the reduction of acetylacetone with sodium borohydride because a yield of 90% has been obtained[234] by this procedure. Thus, acetylacetone in aqueous methanol was refluxed with a slight excess of sodium borohydride for 20 min. To avoid the lengthy extraction procedure previously used (involving alternate base treatment to give

an alkaline solution and acidification, in between a large number of extractions) an alternative method of isolation was developed. The diol product was found to be too water-soluble to be obtained by normal extraction from aqueous solution and continuous extraction of the acidified reaction mixture resulted in product, contaminated with borates, which could not be purified by distillation. Evaporation of the solvents from the reaction mixture was found to yield quantitatively white needle-shaped crystals of (presumably) a borate derivative. This compound was dissolved in water and crude diol was obtained in a quantitative yield by means of a continuous extraction. In order to get rid of borate impurity, the crude mixture was acetylated with acetic anhydride and pyridine. Pentane-2,4-diol was isolated as its diacetate, in a pure state in a yield of 82% (two components present by v.p.c.). It was originally intended to hydrolyse pentan-2,4-diol diacetate and attempt the separation of the *meso*- and *rac*-components of the diol itself. However, a review of the literature indicated that a *meso*-/*rac*-pentane-2,4-diol mixture could be separated only *via* certain derivatives. The mono-p-bromobenzenesulphonate esters separate on an alumina column[235] and the cyclic sulphite esters have been separated by fractional distillation through a four-foot long vacuum jacketed column packed with glass helices[234] (although the *meso*-ester is impure). 2,4-Dichloropentanes have been resolved by vapour-phase chromatography on a dioctylphtalate column[236]. None of these methods looked convenient so it was decided to attempt separation of the pentan-2,4-diol diacetates.

Although small amounts of *meso*- and *rac*-pentan-2,4-diol diacetates could have been obtained by preparative vapour-phase chromatography, separation by distillation was attempted in the hope that rather larger amounts could be obtained. Fractions of pentan-2,4-diol acetate collected during its distillation through a column of glass

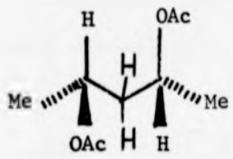
helices were examined by vapour-phase chromatography, but no separation of isomers was achieved.

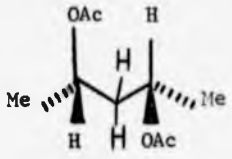
A spinning band distillation was carried out at 10 mmHg. Although most of the signals in the 220 MHz ^{1}H n.m.r. spectrum of *meso*- and *rac*- pentane-2,4-diol diacetates were similar, the signals due to the methylene group of each isomer were diagnostically different. One isomer (lower boiling fraction) was observed to give a symmetrical triplet with a coupling constant of 5.7 Hz. This is consistent with the signal expected from the two enantiomers of *rac*-pentane-2,4-diol diacetate (which give identical spectra). Both methylene protons are affected by a nearby acetyl group and a proton (Scheme 7.3) and therefore are in identical magnetic environments and consequently undergo identical coupling with the two neighbouring methine protons. The methylene group of the other isomer of pentane-2,4-diol diacetate (high boiling fraction) was found to resonate as two multiplets separated by 0.31 p.p.m. This is diagnostic of the *meso*-isomer because its methylene protons can never be formally equivalent, one proton being close to two acetyl groups and the other near to two protons. The methylene protons therefore exhibit different chemical shifts and undergo different coupling with the neighbouring methine protons.

The above argument assumes that facile rotation about the C-C bonds of *meso*- and *rac*-pentane-2,4-diol diacetate is permitted at room temperature (as is almost certainly the case). Therefore, the different conformations, adopted by the diacetates will be averaged out and the ^{1}H n.m.r. signal for their methylene groups will be entirely due to the configurations at their two asymmetric centres.

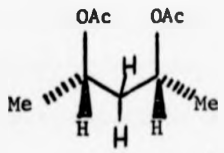
The identification of the isomers was confirmed when samples of *meso*- and *rac*-pentane-2,4-diol diacetate were hydrolysed. The resulting diol isomers were identified by comparison of their i.r.

SCHEME 7.3





(R.R) and (S.S)-Enantiomers
(Exhibiting Identical n.m.r. Signals)
with the Methylene Protons in
Identical Environments



Meso-pentane-2,4-diol Diacetate
with the Methylene Protons in
Different Environments

spectra with those rigorously assigned in the literature[237]. *Meso*-pentane-2,4-diol possesses a characteristic absorption of medium strength at 840 cm^{-1} which is absent in the i.r. spectrum of the *rac*-isomer.

It is interesting to note that the ^1H n.m.r. spectra of *meso*- and *rac*-pentane-2,4-diol exhibited the same characteristics of the methylene protons as their diacetates. However, the difference between the chemical shifts of the two resonances from the methylene protons of the *meso*-isomer was only 0.09 p.p.m.

7.8 A METHOD OF ANALYSIS OF THE REDUCTION PRODUCTS OF 4-HYDROXY-2-OXOPENTANE

Direct analysis of mixtures of *meso*- and *rac*-pentane-2,4-diol from 4-hydroxy-2-oxopentane reduction was shown not to be possible because samples of the diols decomposed, probably by dehydration, under the conditions (v.p.c.) needed for their separation. *Meso*- and *rac*-pentane-2,4-diol possess different ^1H n.m.r. spectra, but the composition of an isomeric mixture cannot be determined by this means, because the methylene proton resonances of each isomer overlap, resulting in a broad signal which cannot be integrated accurately.

An attempt to separate signals in the ^1H n.m.r. spectrum of *meso*- and *rac*-pentane-2,4-diol using the optically active shift reagent europium(III) *tris*-3-trifluoromethylhydroxymethylene-d-camphorate failed, because the large amount of the lanthanide complex needed to achieve sufficient shifts of resonances caused loss of resolution.

Analysis of *rac/meso*-pentane-2,4-diol mixtures by vapour-phase chromatography of their diacetate derivative was ruled out when an experiment showed that isomerisation may occur during the acetylation reaction. When equal amounts of *meso*- and *rac*-pentane-2,4-diol diacetate were mixed together, hydrolysed and reacetylated the isomeric ratio of the product was somewhat removed from unity.

A method of analysis of the reduction products of 4-hydroxy-2-oxopentane was suggested by recent work in which isomers of monosaccharides, including anomeric pairs, were separated by high performance liquid chromatography of their benzoate derivatives[238]. Thus, *meso*- and *rac*-pentane-2,4-diols were reacted with an excess of benzoyl chloride in pyridine. Excess benzoyl chloride was removed by its decomposition with an equimolar amount of water during work-up to form water-soluble benzoic acid and pyridinium hydrochloride. This avoids formation of benzoic anhydride which is obtained when the reaction is carried out in the absence of pyridine and the reaction mixture is poured into a large excess of water[239]. Crude pentane-2,4-diol dibenzoates were purified by recrystallisation from ethyl acetate to give large white needle-shaped crystals of pure *meso*- and *rac*-isomers.

The ^{1}H n.m.r. spectrum of a mixture of *rac*- and *meso*-pentane-2,4-diol dibenzoate showed that the methylene signal of the *rac*-isomer was between and well separated from the two resonances of the *meso*-isomer (0.53 p.p.m. apart due to the large effect of the benzoate group).

These signals could be easily integrated to give an accurate isomeric ratio, thus making the use of high performance liquid chromatography unnecessary. A test of the accuracy of this analytical method was carried out. The ^{1}H n.m.r. spectrum of a solution of exactly equal amounts of *meso*- and *rac*-pentane-2,4-diol dibenzoate in deuterochloroform was integrated ten times and the average ratio of *meso*/*rac*-isomers was 1/1.004.

7.9 REDUCTIONS OF 4-HYDROXY-2-OXOPENTANE

Rac-4-hydroxy-2-oxopentane was reduced in dry THF by slightly more than one mole equivalent of lithium aluminium hydride. The crude pentane-2,4-diol, isolated in almost quantitative yield, was benzoylated.

The product was fairly pure and its isomeric composition was determined without further purification. (A purification step may result in the preferential isolation of one isomer.) The ^{1}H n.m.r. spectrum of the pentane-2,4-diol dibenzoate was integrated ten times and the averaged ratio of *meso/rac*-isomers determined as 1/1.96, respectively.

Reduction of 4-hydroxy-2-oxopentane with an equimolar amount of sodium borohydride in methanol gave, after ether extraction of the reaction mixture, a product heavily contaminated with borate esters. This was overcome by evaporating the reaction mixture to dryness and adding an equal amount of sorbitol, a high boiling polyhydroxy compound which readily forms borate complexes, to the solid borate complex obtained. When this mixture was heated under vacuum pure pentane-2,4-diol distilled out in a nearly quantitative yield. The ^{1}H n.m.r. spectrum of the dibenzoate of this product was integrated ten times and the *meso/rac*-isomer ratio determined as 1.5/1, respectively. This is in fair agreement with an estimate (2/1) in the literature.[234]

4-Hydroxy-2-oxopentane was reduced by a suspension of lithium diethoxy aluminohydride in THF, prepared by the addition of two mole equivalents dry ethanol to a suspension of lithium aluminium hydride in THF. The reaction was worked up as for normal lithium aluminium hydride reduction to give a good yield of pentane-2,4-diol. The ratio of *meso/rac*-isomers in this product was determined in the usual way as 1.7/1, respectively.

7.10 CONCLUSION

Experiments with the reduction of 4-hydroxy-2-oxopentane have shown:

(a) Reduction with sodium borohydride favours production of *meso*-pentane-2,4-diol, whereas the use of lithium aluminium

hydride favours formation of *rac*-pentane-2,4-diol.

(b) The substituents of diethoxy lithium aluminium hydride exert a significant influence on the ratio of *meso*- and *rac*-pentane-2,4-diol.

Control over the stereochemistry of the reduction of 4-hydroxy-2-oxopentane has been demonstrated. The groundwork in this area is complete and it may be possible to achieve highly stereoselective reductions and hence, the easy preparation of pure diastereioisomers of pentane-2,4-diol.

THE SYNTHESIS OF *Rac*-1-(2'-HYDROXYPROPYL)CYCLOHEXA-1,4-DIENE - AN INTERMEDIATE IN THE SYNTHESIS OF 8-METHYLLIPOIC ACID

Although (R)- and (S)-8-methyllipoic acid were to be synthesised from (R)- or (S)-propylene oxide, respectively and phenyllithium (Chapter 2), preliminary experiments were conducted with *rac*-1-phenyl-2-propanol which was available*. This compound underwent Birch reduction readily to give 1-(2'-hydroxypropyl)cyclohexa-1,4-diene in 84% yield. The unsubstituted double bond of this product was successfully selectively reduced with hydrazine hydrate and hydrogen peroxide, but the conditions were not optimised and a low yield (40%) of *rac*-1-(2'-hydroxypropyl)cyclohex-1-ene was obtained.

No further work was done on this route because of lack of time.

---

*Prepared by D. R. Hall, University of Warwick, Coventry

EXPERIMENTAL

Rac-1-(2'-hydroxypropyl)cyclohexa-1,4-diene

dl-1-Phenyl-2-propanol (5 g, 0.037 mol) was dissolved in ethanol (11.3 ml) and the solution was transferred to a 3-necked r.b. flask fitted with stopper, acetone/dry-ice condenser protected from moisture with a drying tube,and a gas inlet. A single necked r.b. flask was connected to the gas inlet by means of a cone to rubber adapter and nylon tubing. Liquid ammonia (50 ml) and two small lumps of sodium were added to the single necked flask which was then warmed with a gentle Bunsen burner flame and dry ammonia was distilled into the reaction flask. When the distillation had ceased, the gas inlet was replaced with a stopper and the sodium in the single necked flask was carefully decomposed by the addition of iso-propanol. Clean sodium (2.11 g, 0.092 mol) was cut into small pieces under pentane which were then added one by one to the reaction flask replacing the stopper after each addition. The solution turned first blue, then a white precipitate formed. After the addition was complete the mixture was left to reflux at room temperature for 10 mins. during which time it turned completely white. The acetone/dry-ice condenser was then replaced with an air condenser and the ammonia left to evaporate overnight whilst the white solid turned red/brown. Water (35 ml) was then carefully added down the condenser to give a brown solution which was extracted with pentane (3 x 50 ml). The combined extracts were dried (anhydrous magnesium sulphate) and evaporated to give a yellow residue which was distilled yielding a colourless oil (3.77 g, 84.23%) b.p. 102-104°, 15 mmHg, pure by v.p.c. (20% Degs on chromosorb, 170°).

N.m.r. (60 MHz, CCl_4, TMS): 1.09 (d, 3 H, CH_3-), 1.99 (d, 2 H, $-CH_2$), 2.28 (brs, 1 H, OH), 2.60 (brs, 4 H, 2 x CH_2 of ring), 7.79 (m, 1 H, HOCH-), 5.47 (brs, 1 H, α C=CH of ring), 5.60 (brs, 2 H, 2 x β C=CH of ring).

Pentane-2,4-diol

To a solution of acetyl acetone (19.01 g, 0.189 mol) in methanol (50 ml) cooled to -12° (ice/salt bath) was added sodium borohydride (5.19 g, 0.14 mol) in water (50 ml) containing sodium hydroxide (0.097 g, 0.0025 mol). The addition was exothermic and carried out over 2 h, whilst the temperature was kept below 10°C. The mixture was refluxed for 20 min. and then evaporated to dryness *in vacuo* to give a mass of white crystals. The solid material was taken up in water (70 ml) and continuously extracted with dichloromethane overnight. After this time the organic phase was separated, dried (anhydrous magnesium sulphate) and evaporated to give a colourless oil of pentane-2,4-diol containing borate ester impurity.

Pentane-2,4-diol diacetate

The crude product obtained above (13.14 g) was added to acetic anhydride (30.6 g, 0.3 mol) and conc. H_2SO_4 (0.5 ml). The mixture was refluxed for 1 h then allowed to cool and then added to water (150 ml). The aqueous mixture was extracted with dichloromethane (3 x 150 ml). The extracts were combined and washed with hydrochloric acid (2 M) and then dried (anhydrous magnesium sulphate). Removal of the solvent afforded a yellow residue which was distilled to yield a colourless oil, (19.46 g, 82%), b.p. 92-94°, 15 mmHg. The oil was examined by v.p.c. (20% Degs on chromosorb W, 150°) and gave two peaks .

Separation of isomers of pentan-2,4-diol diacetate

Pentane-2,4-diol diacetate (16.28 g, 0.086 mol) was placed in a r.b. flask which was then connected to a spinning band distillation apparatus and pumped to 10 mmHg. The flask was heated with an oil bath at 120°. The temperature was adjusted to 82° at the bottom of the column, 80° in the middle and 78° at the top. These conditions gave a reflux rate of 16 drops per min. The collected material was continually examined by v.p.c. (20% Degs on chromosorb W, 150°). The results of the analysis are given in Table 7.1. The total recovery of material was 70%.

N.m.r. *meso*-isomer ($CDCl_3$, TMS): 1.23 (d, 6 H, 2 x $HCC\underline{H}_3$), 1.67 and 2.96 (m's, 1 H each, $-C\underline{H}_2$), 2.03 (s, 6 H, 2 x $OCOC\underline{H}_3$), 4.98 (m, 2 H, 2 x $\underline{H}COAc$). b.p. 69°, 8 mmHg.

Rac-isomer ($CDCl_3$, TMS): 1.22 (d, 6 H, 2 x $HCC\underline{H}_3$), 1.75 (t, 2 H, $-C\underline{H}_2$), 2.05 (s, 6 H, 3 x $OCOC\underline{H}_3$), 4.98 (m, 2 H, 2 x $\underline{H}COAc$). b.p. 64°, 8 mmHg.

Hydrolysis of *rac*- and *meso*-pentane-2,4-diol diacetate

Meso-pentan-2,4-diol diacetate (fraction 6) (1.0 g, 5.32 mmol) was dissolved in methanol (10 ml) and sodium methoxide in methanol (1.32 ml, 0.33 M) added. The mixture was allowed to stand at r.t. for 24 h. Amberlite resin (IRC 50, 90 mg) was then added to the solution and the mixture was stirred for 15 min. The solid material was filtered off and the filtrate evaporated to yield *meso*-product (0.60 g, 56%). b.p. 74°, 5 mmHg.

The procedure was repeated with *rac*-pentane-2,4-diol diacetate (fraction 1), to give *rac*-pentane-2,4-diol (0.54 g, 53.2%). b.p. 75°, 5 mmHg.

I.r. *rac*-pentane-2,4-diol (film): 3325 (br.s), 2960 (s), 2925 (s), 1455 (m), 1410 (m), 1370 (s), 1310 (m), 1265 (sh), 1215 (w), 1150 (m), 1120 (s), 1040 (m), 1000 (m), 960 (m), 920 (m), 880 (w), 830 (m), 790 (w).

Lit. i.r.[12]: 3325, 2960, 2925, 1450, 1410, 1370, 1310, 1265, 1215, 1150,

TABLE 7.1

Analysis of fractions from spinning band distillation of pentan-2,4-diol diacetate by v.p.c.

Fraction	b.p./^{o}	weight/g	Isomer ratio *meso/rac*	Purity main/% isomer	% Yield
1	72	0.20	0:1	100	1.2
2	72	4.51	1:11	91	27.7
3	72-74	1.17	1:7	85	7.1
4	74	0.69	1:6.3	84	4.2
5	76	1.61	4:1	75	9.9
6	76	1.84	16.4:1	94	11.3
7	78	1.43	42:1	98	8.7

1040, 1000m 960, 920, 880, 830, 790.

I.r. *meso*-pentane-2,4-diol (film): 3345 (brs), 2960 (s), 2925 (s), 1455 (m), 1420 (m), 1375 (s), 1320 (m), 1265 (sh), 1215 (w), 1160 (s), 1120 (s), 1040 (m), 1000 (m), 955 (m), 920 (m), 885 (w), 840 (w), 825 (m).

Lit. i.r.[12]: 3340, 2960, 2925, 1455, 1420, 1375, 1320, 1265, 1215, 1160, 1120, 1040, 1000, 955, 920, 885, 840, 825.

N.m.r. *meso*-pentane-2,4-diol (CCl_4, TMS): 1.16 (d, 6 H, 2 x $-C\underline{H}_3$), 1.45 and 1.67 (m's, 1 H each, $-C\underline{H}_2-$), 3.96 (p, 2 H, 2 x $\underline{H}COH$), 4.64 (brs, 2 H, 2 x $-O\underline{H}$).

N.m.r. *rac*-pentane-2,4-diol (CCl_4, TMS): 1.14 (d, 6 H, 2 x $-C\underline{H}_3$), 1.54 (t, 2 H, $-C\underline{H}_2-$), 3.96 (p, 2 H, 2 x $-C\underline{H}$), 4.94 (brs, 2 H, 2 x $O\underline{H}$).

Reacetylation of pentane-2,4-diol

Equal amounts of pure *meso*- and *rac*-pentane-2,4-diol diacetate were taken up in ether and the solutions mixed together. The solvent was evaporated from the mixture to yield an equimolar mixture of diacetate which was hydrolysed using the procedure given previously. The resulting equimolar mixture of *meso*- and *rac*-pentane-2,4-diol was subjected to acetylation using the procedure used earlier. The product was examined by v.p.c. and the ratio of *meso*- and *rac*-isomers was found to have changed from 1:1 respectively for the starting diacetate to 0.45:1.

4-Hydroxy-2-oxopentane

Acetaldehyde (53.2 g, 0.9 ml) was added dropwise, simultaneously with a solution of sodium hydroxide (7.42 g, 0.18 mol) in water (80 ml) to a mixture of acetone (416 ml, 910 mol) and water (532 ml), over 1½ h at -5°. The mixture was then stirred for 1 h at r.t. and then it was

extracted with dichloromethane (5 x 100 ml). The combined organic fractions were dried (anhydrous magnesium sulphate) and evaporated to yield a slightly green oil (52.4 g). The ^{1}H n.m.r. spectrum of the product indicated that it consisted of 43% 4-hydroxy-2-oxopentane and 57% 4-hydroxy-4-methyl-2-oxopentane.

N.m.r. (60 MHz, CCl_4, TMS): 4-hydroxy-4-methyl-2-oxopentane, 1.17 (s, 6 H, 2 x $\underline{CH_3}OH$), 1.98 (s, 3 H, $\underline{CH_3}CO$), 2.39 (s, 2 H, $-\underline{CH_2}-$), 3.34 (br.s, 1 H, $-\underline{OH}$).

4-Hydroxy-2-oxopentane, 0.98 (d, 3 H, $\underline{CH_3}CH-$), 1.99 (s, 3 H, $\underline{CH_3}CO$), 2.35 (d, 2 H, $\underline{CH_2}CH-$), 3.50 (br.s, 1 H, $-\underline{OH}$).

4-Hydroxy-2-oxopentane and 4-hydroxy-4-methyl-2-oxopentane were separated by a spinning band distillation as follows: the mixture (45 g) was weighed into a r.b. flask which was then connected to a spinning band distillation apparatus which was then put under vacuum (15 mmHg). The flask was heated in an oil bath at 68°. The column temperature was adjusted to 62° at the bottom, 60° in the middle and 58° at the head to give a reflux rate of 16 drops per min. The collected material was examined by ^{1}H n.m.r. spectroscopy at 20 min. intervals. The results are given in Table 7.2.

Dibenzoates of *meso*- and *rac*-pentane-2,4-diol: General procedure

A sample of pentane-2,4-diol (200 mg, 1.9 mmol) was dissolved in pyridine (2 ml) at r.t. After 10 min. the solution was cooled to 4° and benzoyl chloride (0.800 g, 5.7 mmol, 1 mol equivalent per hydroxy group + 1 mol equivalent excess) was added in three portions over 1 h with periodic shaking whilst the temperature was maintained at 4°. The reaction was left at 4° overnight followed by 2 h at r.t., prior to the addition of pre-cooled distilled water (68 g, 3.8 mmol) with ice cooling in an ice bath. After a further 1 h

TABLE 7.2

SPINNING BAND DISTILLATION OF MIXTURE OF 4-HYDROXY-2-OXOPENTANE AND 4-HYDROXY-4-METHYL-2-OXOPENTANE

Fraction	bp/^{o}	Weight/g	Purity 4-hydroxy methyl 2-pentanone	% recovery
1	54	4.01	98	8.8
2	54	3.76	98	8.3
3	56	1.77	86	3.9
4	58	2.28	85	5.0
5	60	4.72	50	10.4
6	61	2.78	30	6.1
7	62	3.89	15	8.5
8	62	6.29	1	13.8
TOTAL RECOVERY				65

at r.t. dichloromethane (2 ml) was added and the resulting mixture was cooled to 4° and washed with pre-cooled sulphuric acid (2 x 2 ml, 1 M). The organic layer was separated and dried (anhydrous sodium sulphate) and evaporated. A pale brown solid was obtained which was recrystallised from boiling ethyl acetate 140-60° petroleum ether (1/1) to give white needle shaped crystals.

From pure *meso*-pentane-2,4-diol (200 mg) white crystals (0.57g, 96%) of the dibenzoate was obtained.

N.m.r. ($CDCl_3$, TMS): 1.42 (d, 6 H, 2 x $-CH_3$), 1.92 (m, 1 H) and 2.45 (m, 1 H) due to each proton of the methylene group, 5.45 (m, 2 H, 2 x -CH), 7.40-8.02 (m's, 10 H, 2 x aromatic rings).

From pure *rac*-pentane-2,4-diol (200 mg) white crystals (0.60 g, 98%) of dibenzoate were obtained.

N.m.r. ($CDCl_3$, TMS): 1.43 (d, 6 H, 2 x $-CH_3$), 2.02 (t, 2 H, $-CH_2-$), 5.33 (m, 2 H, 2 x -CH), 7.38-7.99 (m's, 10 H, 2 x aromatic rings).

Reduction of 4-hydroxy-2-oxopentane with sodium borohydride

4-Hydroxy-2-oxopentane (0.2 g, 1.96 mmol) was dissolved in water (2.5 ml). The resulting solution was cooled in an ice-slush bath and sodium borohydride (0.05 g, 1.3 mmol) in water (2.5 ml) was added dropwise over 10 min. The reaction mixture was then allowed to stand at r.t. overnight. After this time, the water was evaporated, *in vacuo*, to leave white crystals of borate complex. To this solid material was added an equal amount of sorbitol and the mixture was then heated to 100° under vacuum (0.1 mmHg) in a Kugelröhr apparatus until an oil ceased to distil out. Comparison of the ^1H n.m.r. spectrum of the oil with those for *meso*- and *rac*-pentane-2,4-diol showed that it consisted of a mixture of the isomers of pentane-2,4-diol. The amount of each diastereoisomer could not be determined by integration due to a lack of separation of the relevant peaks.

The diol mixture (200 mg, 1.9 mmol) was converted to its dibenzoate using the procedure described earlier. An oil (0.58 g, 96%) was obtained and no attempt was made to purify it by recrystallisation in case one isomer crystallised preferentially over the other, leading to a misleading isomer ratio. The ^{1}H n.m.r. spectrum of the product showed it to consist of pure dibenzoate. The peaks due to *meso*- and *rac*-isomers were well separated and the area determined by integration. An average of 10 integral values gave the ratio of *rac/meso*-isomers as 1/1.5 respectively.

Reduction of 4-hydroxy-2-oxopentane with lithium aluminium hydride

4-Hydroxy-2-oxopentane (0.2 g, 1.96 mmol) was dissolved in dry THF (5 ml) and added dropwise under nitrogen to a suspension of lithium aluminium hydride (0.1 g, 2.96 mmol) in dry THF (5 ml). When the addition was finished the mixture was refluxed overnight then allowed to cool to r.t. whereupon water (0.5 ml) was carefully added. The white precipitate that formed was filtered off and washed with ether (15 ml). The filtrate and washings were combined, dried (anhydrous magnesium sulphate) and evaporated to give a colourless oil (0.196 g, 96.5%). The ^{1}H n.m.r. spectrum was consistent with that of a mixture of *meso*- and *rac*-diol. The diol mixture was converted to a mixture of dibenzoates using the procedure described earlier. An ^{1}H n.m.r. spectrum of the oil obtained showed the product to consist of fairly pure dibenzoate. The *meso*-/*rac*-isomer ratio was measured by an average of 10 integrations of the relevant peak areas as 1.96/1 *rac*-/*meso*-.

Reduction of 4-hydroxy-2-oxopentane with lithium diethoxy aluminohydride

To a suspension of lithium aluminium hydride (100 mg, 2.6 mmol), in dry THF (5 ml) was added dry ethanol (300 µl). After the suspension

had been stirred for 30 min. at r.t. a solution of 4-hydroxy-2-oxopentane (0.2 g, 1.96 mmol) in dry THF (5 ml), was added dropwise over 10 min. The mixture was then refluxed overnight and allowed to cool to r.t. Water (300 µl) was then added to the mixture, followed by 15% sodium hydroxide (300 µl), and more water (900 µl). The resulting white precipitate was filtered off and washed with ether (15 ml). The filtrate and washings were combined, dried (anhydrous magnesium sulphate) and evaporated to give a colourless oil (0.146 g, 72%). The oil was shown by ^{1}H n.m.r. to be a mixture of *meso*- and *rac*-diols. No other products were detected. The oil was converted to pentane-2,4-diol dibenzoate as described previously. An ^{1}H n.m.r. spectrum of the resulting oil showed it to consist of pure *meso*- and *rac*-dibenzoates in a ratio of 1.7/1 respectively.

Experiment to test the accuracy of the isomeric ratio determination of pentane-2,4-diol dibenzoate

Meso-pentane-2,4-diol dibenzoate (0.1 g) and *rac*-pentane-2,4-diol dibenzoate (0.1 g) were weighed into separate flasks. Ether (10 ml) was added to each flask and the resulting solution mixed together to give a 1/1 mixture of the isomers. Peaks due to each isomer in the ^{1}H n.m.r. spectrum of the mixture were integrated ten times and averaged to give a *meso*-/*rac*-ratio of 1/1.004.

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CHAPTER 8

MATERIALS AND METHODS

SOLVENTS

Dry ethanol (and methanol)

To 1 litre of commercial alcohol was added pre-dried magnesium (5g), and a small crystal of iodine. The mixture was heated until the magnesium had completely reacted to form magnesium alkoxide. The dry alcohol was distilled from the mixture and stored over dried 4A molecular sieves.

Dry ether and THF

The commercial 'AnalaR' grade of solvent was distilled from lithium aluminium hydride and stored over dried 3A molecular sieves.

Dry DMSO and Xylene

The commercial 'AnalaR' grade of solvent was dried by storage over 3A molecular sieves.

Ethanol-free chloroform

The commercial 'AnalaR' grade of chloroform was passed through a column of basic alumina (200g/litre chloroform).

CHEMICALS

Purification

All chemicals were of the highest purity commercially available and purified where necessary:

Potassium

20% more potassium than required was placed in a wide-mouthed Erlenmeyer flask and covered with dry xylene containing 1% isopropanol.

The flask was heated on a hot-plate until the potassium melted, when the flask was removed from the heat and swirled gently to cause small spheres of potassium to form. The flask was covered and allowed to cool undisturbed. The resulting 'blue eggs' of potassium were removed with forceps and weighed under paraffin.

Anhydrous magnesium chloride

Commercial magnesium chloride hexahydrate, (BDH), was covered with thionyl chloride in a r.b. flask. The mixture was refluxed (fume cupboard) for 1 hr and the excess thionylchloride was then distilled off. Traces of thionyl chloride were removed from the dry magnesium chloride in a vacuum desiccator (10 mmHg).

1,1,1-tris-(hydroxymethyl)ethane

'Technical' grade (Aldrich) 1,1,1-tris-(hydroxymethyl)ethane was sublimed at 110°, 10 mmHg.

Acetyl chloride

Commercial acetyl chloride (Hopkins and Williams) was refluxed with PCl_5 for several hours to remove traces of acetic acid then distilled. Redistillation from one tenth-volume of quinoline removed dissolved HCl.

Benzoyl chloride

A solution of benzoyl chloride (Aldrich) (300ml) in toluene (200ml) was washed with portions of cold 5% sodium bicarbonate solution (3 x 100ml). The organic layer was separated, dried with calcium chloride and distilled.

Anhydrous magnesium sulphate

Commercial anhydrous magnesium sulphate (Aldrich) was kept in an oven at 110°.

11-Bromoundecanoic acid

'Technical' grade 11-bromoundecanoic acid was recrystallised from ethanol/water (2/1) and dried in vacuo over silica gel.

Preparations

Copper (II) iodide

Copper (II) sulphate (25g, 0.1 mol) was dissolved in water (150ml). To this solution was added, from a burette with continuous rapid stirring, a solution of potassium iodide (36.5g, 0.22 mol) and sodium thiosulphate.$5H_2O$ (28.0g, 0.11 mol) in water (100 ml). When no further precipitation occurred the dense white precipitate was allowed to settle for 15 min then filtered off on a sintered glass funnel, and washed with water (2 x 20ml), ethanol (2 x 20ml) and ether (3 x 20ml). The product was dried in vacuo over P_2O_5.

Lithium shavings

The required amount of lithium was weighed out then hammered into a flat sheet and cut into shavings with scissors, directly into the reduction mixture.

Sodium methoxide in methanol

The calculated amount of sodium was added in small pieces to dry methanol under nitrogen.

Sodium phenoxide

Phenol (4.19g, 0.04 mol) was added to aqueous 1M sodium hydroxide (50ml). After 5 min standing the solution was concentrated and

dichloromethane (50ml) was added. On shaking, white crystals appeared which were filtered off and dried in vacuo. A white powder of sodium phenoxide (5.28g, 99%) was obtained.

INSTRUMENTAL

N.m.r. spectra

Taken on:

i) Perkin Elmer (model R12) 60MHz for ^{1}H

ii) Perkin Elmer (model R34) 220MHz for ^{1}H

Peaks are designated by the chemical shift (δ) in parts per million followed, in brackets, by the multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentuplet, m = multiplet and br = broad peak), the spin-spin coupling constant, J, in Hz (where relevant) and the integration, H, for hydrogens. N.m.r. spectra were taken on the Perkin Elmer (model R34) at 220MHz, unless otherwise stated.

I.r. spectra

Taken with Perkin Elmer (model 257) grating infra red spectrophotometer. Calibration of spectra was done on the 1601.4 peak of the polystyrene spectrum. Peaks are designated by their wave number (cm^{-1}) as strong (s), medium (w), broad (br) and shoulder (sh).

V.p.c. analysis

Carried out with a Perkin Elmer (model F11) gas chromatograph fitted with a flame ionisation detector. Melting points were determined by an electrothermal melting point apparatus in open capillary tubes.

Optical rotations

Measured with a Bendix NPL automatic polarimeter (model 143D) using a 1.00cm x 0.708cm^2 cell. The instrument was calibrated with standard sucrose and mannitol solutions.

U.v. spectra

Taken on a Pye-Unicam (model SP1800) thermostatted ultraviolet spectrophotometer using quartz cells.

METHODS (GENERAL DESCRIPTION)

Glassware

Dried at 110° overnight or flamed and then cooled to room temperature in a desiccator, over P_2O_5.

Moisture-sensitive reactions

Carried out under a blanket of dry nitrogen.

Bulk removal of solvents

Low boiling solvents were removed with a Buchi rotary evaporator at 20°, 10 mmHg and higher boiling solvents were removed with a rotary evaporator at 40°.

Thin-layer chromatography

20cm x 5cm plates and silica gel (Kieselgel 60_{F254}) from Merck were used. The absorbent was slurried in ethyl acetate, the plates prepared by dipping, and activated by heating at 110° for 2 h. The plates were stored over silica gel (self-indicating) in a closed box. Visualisation was carried out either by spraying of the plates with 35% sulphuric acid followed by charring, or by iodine. Results are given in the format:

(solvent systems, method of detection, R_F value)

Absoption column chromatography

Fisons silica gel 80-200 mesh was used. Columns were packed with a slurry of silica gel and solvent and air bubbles removed as the absorbent settled, by gently tapping.

Absoption column chromatography

Fisons silica gel 80-200 mesh was used. Columns were packed with a slurry of silica gel and solvent and air bubbles removed as the absorbent settled, by gently tapping.

ADDENDUM

CRITERIA OF PURITY

ANALYSES

4-(3'-chloropropyl)-1-methyl-2,6,7-trioxabicyclo[2,2,2]octane.

$C_9H_{15}ClO_3$ (MW 206) found C 51.77, H 7.29, Cl 17.71

requires C 52.31, H 7.32, Cl 17.15.

2'-2'-*bis*(hydroxymethyl)propyl 4-chlorobutanoate

$C_9H_{17}ClO_4$ (MW 223) found C 47.59, H 7.70

requires C 48.11, H 7.57.

LITERATURE VALUES OF PHYSICAL DATA

11-bromoundecanoamide - m.p. 87.5°, lit. m.p.[240] 88°.

4-chlorobutyronitrile - b.p. 92-94°, 25 mmHg, lit. b.p.[241] 95-96°, 26 mmHg.

1,1,1-triethoxyethane - b.p. 144°, 760 mmHg, lit. b.p.[168] 144-146°, 760 mmHg.

1-methyl-2,8,9-trioxaadamantane - m.p. 126°, lit. m.p. 126°[242].

γ-butyrolactone - b.p. 82-83°, 10 mmHg, lit. b.p. 89°, 12 mmHg, lit. n.m.r.[244] ($CDCl_3$, TMS) δ 2.40 (m, 4 H, $-CH_2CH_2-$) 4.35 (t, 2 H, CH_2O-), lit. i.r.[245] 3000 (m), 2980 (m), 1765 (v.s.), 1460 (m), 1420 (m), 1380 (m), 1280 (w), 1230 (w), 1160 (v.s.), 1030 (s), 990 (s), 930 (m), 865 (m), 800 (m).

Ethyl 4-chlorobutanoate - b.p. 186-188, 760 mmHg, lit. b.p.[246] 186°, 760 mmHg.

lit. i.r.[247a] 2995 (s), 1740 (s), 1410 (m), 1350 (m), 1320 (m), 1250 (m), 1200 (m), 1140 (m), 1000 (w), 850 (w), 780 (w).

Methyl 4-chlorobutanoate - b.p. 176-177°, 760 mmHg, lit. b.p.[246] 175-176, 760 mmHg, lit. i.r.[247b] 2995 (m), 1740 (s), 1420 (m), 1360 (m), 1310 (m), 1280 (m), 1200 (s), 1160 (m), 1130 (m), 1050 (m), 1000 (w), 880 (w), 780 (w).

1,4-dimethyl-2,6,7-trioxabicyclo[2,2,2]octane - m.p. 83-84° lit. m.p.[167] 105, lit. ^{1}H n.m.r. ($CDCl_3$, TMS) δ 0.81 (s, CH_3), 3.88 (s, CH_2), 1.43 (s, CH_3CO_3).

2-methyl-2-hydroxydecane - b.p. 106°, 760 mmHg, lit. b.p.[248] 108.5, 760 mmHg.

2-acetylbutyrolactone - b.p. 118°, 10 mmHg, lit. b.p.[249] 107-108°, 5 mmHg.

5-chloro-2-oxopentane - b.p. 58-60°, 10 mmHg, lit. b.p.[182] 70-72, 20 mmHg.

2-(3'-chloropropyl)-2-methyl-1,3-dioxolane - b.p. 80°, 760 mmHg, lit. b.p.[147] 84°, 13 mmHg.

2-methyl-2-propyl-1,3-dioxolane - b.p. 42°, 20 mmHg, lit. b.p.[147] 40°, 20 mmHg.

trans-1,4-dihydroxybut-2-ene - b.p. 136-138°, 10 mmHg, lit. b.p.[250] 131°, 13 mmHg.

cis-1,4-dihydroxybut-2-ene - b.p. 135°, 10 mmHg, lit. b.p.[250] 132°, 16 mmHg.

2,2-dimethyl-4-vinyl-1,3-dioxolane - b.p. 85°, 125 mmHg, lit. b.p.[191] 52°, 50 mmHg.

(R)-1,2-dihydroxybut-3-ene - b.p. 70-72°, 10 mmHg, lit. b.p.[251] R,S-1,2-dihydroxybut-3-ene 191-192°, 747 mmHg.

(S)-4- methylthiobutane-1,2-diol b.p. 150-152°, 8 mmHg,

lit. b.p.[216] 100-102.5°, 0.2 mmHg.

Meso-pentane-2,4-diol diacetate b.p. 69°, 8 mmHg,

lit. b.p.[252] 70°, 4 mmHg.

Rac-pentane-2,4-diol diacetate - b.p. 64°, 8 mmHg,

lit. b.p.[252] 62°, 4 mmHg.

4-hydroxy-2-oxopentane - b.p. 62°, 15 mmHg,

lit. b.p.[253] 177°, 760 mmHg.

4-hydroxy-4-methyl-2-oxopentane - b.p. 54°, 15 mmHg,

lit. b.p.[254] 72°, 20 mmHg.

Meso-pentane-2,4-diol - b.p. 74°, 5 mmHg, lit. b.p.[234] 73°, 3 mmHg.

Rac-pentane-2,4-diol - b.p. 75°, 5 mmHg, lit. b.p.[234] 74°, 3 mmHg.

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